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EARLY CARDIOVASCULAR RISK MARKERS AND CARDIAC FUNCTION IN CHILDREN WITH CHRONIC KIDNEY DISEASE

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“Mot det förgångna: tack, till det kommande: ja!”

Dag Hammarsköld

Till min älskade pappa

Karolinska Institutet

Institutionen för klinisk vetenskap, intervention och teknik, Enheten för pediatrik

Early cardiovascular risk markers and cardiac function in children with chronic kidney disease

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Stockholm 2016

ABSTRACT

Children with advanced chronic kidney disease (CKD) have an increased risk of premature death, foremost due to cardiovascular disease (CVD). The cardiovascular (CV) morbidity starts early in the disease process and renal transplanted children (CKD-T) are also at risk.

Aims: The overall aim of this thesis was to study CV morbidity and potential risk factors in pediatric CKD and CKD-T patients. The prevalence of various known biomarkers associated with increased risk of CVD was assessed (Papers I, II and IV). Furthermore, CV morbidity and associated risk factors were analyzed cross-sectionally (Paper II) and longitudinally (Papers III and IV). Associations between potential CV risk factors and CV outcome was explored in order to identify possible predicting factors (Papers III and IV).

Methods: In total 26 Italian (Paper I) and 34 Swedish CKD and 44 CKD-T patients (Paper II) were included. The Swedish cohort was followed prospectively every year for 3 years (Papers III-IV). Fasting blood samples were analyzed in regard to anemia, inflammation, abnormal glucose metabolism, dyslipidemia and altered mineral metabolism. The renal function and blood pressure levels were also assessed. Using echocardiography, the left ventricular mass index (LVMI) and left ventricular (LV) function were examined and, the carotid intima media thickening (cIMT) was further analyzed (Papers II-IV).

Results: Regarding biomarkers of CV risk, the dominant finding was high levels of insulin and insulin resistance (Papers I and II), but the lipid profile and inflammatory status were also altered (Paper II). In addition, high Fibroblast Growth Factor-23 (FGF23) and PTH revealed a disturbed mineral metabolism (Paper IV). Regarding CV morbidity, cardiac remodeling (increased LVMI) and markers of LV diastolic dysfunction were significantly altered in both patient groups, while the cIMT was normal (Papers II and III). Tissue Doppler Imaging (TDI) revealed early signs of LV diastolic dysfunction, present in 7.1% of CKD and 12.5% of CKD-T patients (Paper III). Furthermore, TDI was more sensitive in diagnosing subtle changes in cardiac function compared to conventional pulse wave Doppler (PWD). The most important risk factors for subclinical CVD were a young age, elevated BMI and systolic blood pressure z-scores as well as a low GFR and present albuminuria (Paper III). Increasing blood pressure and BMI over follow-up were also important cardiac risk factors longitudinally (Paper III). Both high FGF23 and low Klotho were associated with a worse LV diastolic function in CKD-T patients (Paper IV).

Conclusion: These results leads to the conclusion that an altered cardiac function and remodeling are a concurrent part of the CKD process, start early in the disease development, and persist after renal transplantation. The findings suggest that children with CKD or CKD-T are at high risk for future CVD where younger patients with elevated BMI and slightly increased blood pressures, as well as present albuminuria, are those at greatest risk, thus indicating targets for future interventions. The role of FGF23 and Klotho in cardiac morbidity is interesting and might be one of the missing pieces in this complicated puzzle of CKD-associated CVD.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following Papers. The Papers will be referred to by their Roman numerals (I-IV).

I. **Ylva Tranæus Lindblad**, Jonas Axelsson, Peter Bárány, Gianni Celsi, Bengt Lindholm, Abdul Rashid Quershi, Alba Carrea, Alberto Canepa.
Hyperinsulinemia and Insulin Resistance, Early Cardiovascular Risk Factors in Children with Chronic Kidney Disease.
Blood Purification 2008; 26: 518-525.

II. **Ylva Tranæus Lindblad**, Jonas Axelsson, Rita Balzano, Georgios Vavilis, Milan Chromek, Gianni Celsi, Peter Bárány.
Left ventricular diastolic dysfunction by tissue Doppler echocardiography in pediatric chronic kidney disease.
Pediatric Nephrology 2013; 28: 2003-2013.

III. **Ylva Tranæus Lindblad**, Georgios Vavilis, Jonas Axelsson, Maria Herthelius, Peter Bárány.
Assessing longitudinal trends in cardiac function among pediatric patients with chronic kidney disease.
Accepted for publication in Pediatric Nephrology.

IV. **Ylva Tranæus Lindblad**, Hannes Olausson, Georgios Vavilis, Ulf Hammar, Maria Herthelius, Jonas Axelsson, Peter Bárány.
The FGF23 and Klotho axis in pediatric chronic kidney disease - a prospective cohort study.
Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ABPM	Ambulatory Blood Pressure Measurement
ACE-I	Angiotensin Converting Enzyme Inhibitor
ADA	American Diabetes Association
ARB	Angiotensin Receptor Blocker
A4C	Apical 4 Chamber
A2C	Apical 2 Chamber
BMI	Body Mass Index
BP	Blood Pressure
CAC	Coronary Artery Calcification
CAKUT	Congenital Anomalies of the Kidney and Urinary Tract
cc-TDI	Color Coded-Tissue Doppler Imaging
CDC	Centers for Disease Control and Prevention, U.S.
cIMT	Carotid Intima Media Thickness
CKD	Chronic Kidney Disease
CKD-T	Chronic Kidney Disease in Renal Transplant Recipient
CKiD	Chronic Kidney Disease in Children Prospective Cohort Study
CKD-MBD	Chronic Kidney Disease - Mineral Bone Disorder
CMR	Cardiac Magnetic Resonance
CRS	Cardio-Renal Syndrome
CV	Cardiovascular
CVD	Cardiovascular Disease
DT	Deceleration Time
EDTA	European Dialysis and Transplant Association
EDV	End-Diastolic Volume
EF	Ejection Fraction
ELISA	Enzyme-Linked Immunosorbent Assay
ESCAPE	Effect of Strict Blood Pressure Control and ACE-inhibition on the Progression of Chronic Renal Failure in Pediatric Patients Clinical Trial

ESPN/ERA	European Society of Pediatric Nephrology/ European Renal Association
ESRD	End Stage Renal Disease
ESV	End Systolic Volume
FGF23	Fibroblast Growth Factor-23
FSH	Follicle Stimulating Hormone
GEE	Generalized Estimating Equations
GFR	Glomerular Filtration Rate
GH	Growth Hormone
HCO_3	Bicarbonate
HD	Hemodialysis
HDL	High-Density Lipoprotein
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance Index
Hs-CRP	High-sensitive C-Reactive Protein
HUS	Hemolytic Uremic Syndrome
ICAM	Intracellular Adhesion Molecule
ICC	Intra Class Correlation
IFG	Impaired Fasting Glucose
IGF-1	Insulin-like Growth Factor-1
IGFBP3	Insulin-like Growth Factor Binding Protein 3
IGT	Impaired Glucose Tolerance
IL-6	Interleukin-6
IMT	Intima Media Thickness
KDIGO	Kidney Disease Improving Global Outcomes
LA	Left Atrium
LD	Lumen Diameter
LDL	Low-Density Lipoprotein
LH	Leuteinizing Hormone
LVDD	Left Ventricular Diastolic Dysfunction
LVIDd	Left Ventricular Internal Diameter in Diastole
LVH	Left Ventricular Hypertrophy

LVMI	Left Ventricular Mass Index
MMF	Mycophenolate Mofetil
NAPRTCS	North America Pediatric Renal Trials and Collaborative Studies
NFAT	Nuclear Factor of Activated T-cells
NFK-KDOQI	The National Kidney Foundation – Kidney Disease Outcomes and Quality Initiative
NODAT	New Onset Diabetes After Transplantation
OGTT	Oral Glucose Tolerance Test
PD	Peritoneal Dialysis
Pmarp	Per Million Age Related Population
PTH	Parathyroid Hormone
PTWd	Posterior Wall Thickness in Diastole
PWD	Pulse Wave Doppler
PW-TDI	Pulse Wave-Tissue Doppler Imaging
RCT	Randomized Controlled Trials
RRT	Renal Replacement Therapy
RWT	Relative Wall Thickness
SDS	Standard Deviation Score
SHBG	Sex-Hormone Binding Globulin
SI-unit	International System of Units
SWTd	Septal Wall Thickness in Diastole
UC	Uremic Cardiomyopathy
UNX	Unilateral Nephrectomy
USRDS	United States Renal Data System
UTI	Urinary Tract Infection
VCAM	Vascular Cell Adhesion Molecule
VLDL	Very Low Density Lipoprotein

1 GENERAL INTRODUCTION

Chronic kidney disease (CKD) refers to a condition related to irreversible kidney damage that can further progress to end-stage renal disease (ESRD). CKD affects 13.6% of the U.S. population, and in 2013 a total number of 661,648 people in the U.S. were diagnosed with ESRD, which indicates the need for renal replacement therapy (RRT); i.e. dialysis or renal transplantation[1]. ESRD is a devastating disorder associated with excessive mortality and morbidity rates, but these risks are already increased in early stage CKD. Renal transplantation improves both morbidity and survival rates, but the risks for disease and early death still remain higher than in the general age-matched population[2].

While CKD is not as common in children as in adults, the prevalence of RRT in children has increased in the last decades. A total number of 9,921 children with RRT in the U.S. in 2013 and 3,595 children aged 0-14 years with RRT in Europe in 2011, indicates that this is a significant medical problem[1 3].

The first pediatric renal transplant was performed over 50 years ago, and there have been significant improvements in post-transplant survival and care since then. In addition, advances in surgical techniques have allowed successful transplantations to be performed in smaller (and younger) children. Still, as with adults, the risk of morbidity and mortality in patients after renal transplantation is not normalized, with a major cause being attributed to cardiovascular disease (CVD).[4]

As early as the mid 1980´s, reports of an increased prevalence of cardiovascular (CV) complications in children and adolescents with ERSD were published. The European Dialysis and Transplant Association (EDTA) reported data from 1985 suggesting that 41% of all deaths in pediatric patients were attributable to CV causes[5]. In the mid-1990´s it became clear that CV alterations linked to CVD and mortality began even before ESRD[6], possibly as an attempt to adapt to the hemodynamic and biological derangements already present in early CKD. In the most recent years, several efforts have been made to identify important risk factors for CKD-associated CVD in children. As these children often lack many of the traditional CV risk factors common in adult CKD, the latest research has focused on non-traditional; i.e. uremic related risk factors.

In the last decade new echocardiographic techniques have enabled the analysis of subtle subclinical changes in cardiac geometry and function, shown to predict future CV events and death in both the general population and adult CKD patients[7 8]. However, long-term studies of subclinical CV morbidity in pediatric CKD are scarce. The main aim of this thesis is thus to longitudinally study cardiac and vascular health in pediatric CKD using modern methods, and to identify predictive risk factors which might be possible targets for future preventive interventions.

2 BACKGROUND

2.1 KIDNEY PHYSIOLOGY

2.1.1 The healthy kidney

The kidney is a complex organ central to many tasks in the body. It filters blood to remove toxins, excess salts and waste products, balances water and pH, and regulates blood pressure. The functional parts of the kidneys are called nephrons. There are about one million nephrons in each kidney, and each nephron consists of glomeruli, capillaries, arterioles and tubules, Figure 1. At the glomerular basement membrane within Bowman´s capsule, blood is filtered to form primary urine which then enters the tubular network of the nephron. This network is divided into four segments; the proximal convoluted tubule (which reabsorbs important nutrients, ions and water), the loop of Henle (which concentrates the urine by removing water), the distal convoluted tubule (which regulates potassium, sodium, calcium and pH) and the collecting duct (which collects the urine and regulates the final sodium concentration).

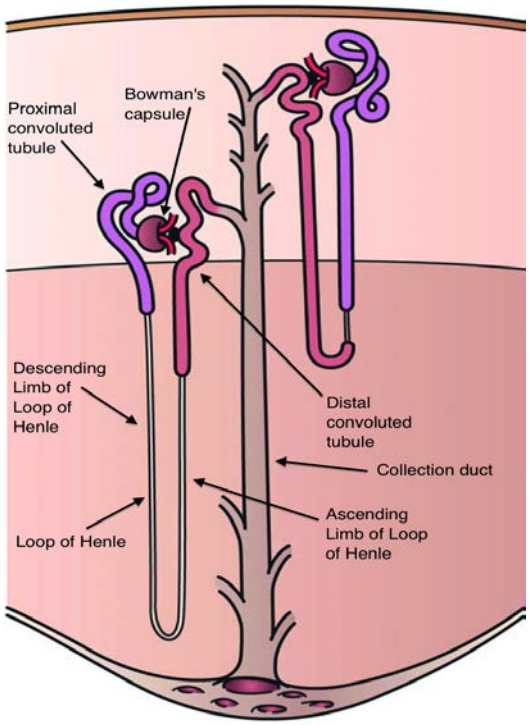

Figure 1. Schematic figure of the nephron.
Image created by Holly Fischer at h*ttps://commons.wikimedia.org/wiki/File:Kidney_Nephron.png*
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The kidneys regulate blood pressure in multiple ways, including the renin-angiotensin aldosterone system (RAAS). This is a hormone system that acts to modulate blood pressure and fluid balance via constriction of vessels and retention of sodium and water. The kidneys

also behave as an endocrine organ by producing the hormone erythropoietin, which regulates the production of red blood cells. Additionally, the kidneys have an important role in calcium-phosphorus metabolism in the body. In order to stabilize calcium levels, the kidneys reabsorb calcium in the tubular system. If this reabsorption is decreased (like in CKD) a compensatory increase in parathyroid hormone (PTH) occur which stimulates vitamin D to facilitate absorption of calcium from the small intestine and increase calcium and phosphate efflux from bone. These functions are progressively impaired in CKD, and as renal function deteriorates, waste products accumulate in the body, the blood pressure is elevated, anemia develops, the bones are demineralized and the risk for CVD increases.

2.1.2 Glomerular filtration rate

Renal function is partly assessed by glomerular filtration rate (GFR). This estimates how much fluid is filtered through the glomeruli each minute, and normal values are corrected for age, body size (surface area) and gender. It is the sum of filtration rates in all nephrons that is assessed and GFR gives an estimation of the number of functioning nephrons. GFR is measured from clearance of a filtration marker; e.g inulin or iohexol, the latter being most commonly used in Sweden. Iohexol is a water-soluble contrast agent that is injected intravenously, is not metabolized and is thus filtered freely in the glomeruli, meaning that the rate of elimination of plasma iohexol can be used to assess GFR. A normal GFR is ≥90-130 ml/min/1.73m² in children above two years of age.

2.1.3 Definition of CKD and CKD staging

CKD is a condition characterized by a gradual but irreversible loss of kidney function over time. Historically, there has been no common definition or classification of CKD. In 2003 the American National Kidney Foundation´s Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) published a guideline for CKD that included a staging system applicable to children[9]. The last revision was published in the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 guidelines, Figure 2[10].

The KDIGO 2012 system diagnoses pediatric CKD if either: 1) GFR is less than 60 ml/min/1.73m² over 3 months or 2) GFR over 60 ml/min/1.73m² and evidence of structural damage or other markers of functional kidney abnormalities including proteinuria, albuminuria, renal tubular disorders or pathologic abnormalities detected by histology or by imaging. The level of GFR and albuminuria are categorized to yield different CKD stages (1-5). The cut-offs for albuminuria are based on urinary albumin to creatinine ratio.[10]

The term end-stage renal disease (ESRD) refers to a GFR less than 15 ml/min/1.73m², i.e. CKD stage 5. At this level the patient is usually referred for renal replacement therapy (RRT; dialysis or renal transplantation) and these patients are commonly also classified as ESRD patients. The term CKD-T is used to indicate that the patient has a renal transplant[9]. In this thesis, CKD is used to denote patients without RRT. Also, RRT signifies patients treated with dialysis or a renal transplant, while CKD-T, peritoneal- or hemodialysis specifies the type of RRT where necessary.

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012

GFR categories (ml/min/ 1.73 m²) Description and range			Persistent albuminuria categories Description and range		
			A1	A2	A3
			Normal to mildly increased	Moderately increased	Severely increased
			<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
G1	Normal or high	≥90			
G2	Mildly decreased	60-89			
G3a	Mildly to moderately decreased	45-59			
G3b	Moderately to severely decreased	30-44			
G4	Severely decreased	15-29			
G5	Kidney failure	<15			

Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

Figure 2. CKD staging according to KDIGO 2012 guidelines. GFR is divided into 5 stages (G1-5, where G3 is further divided into G3a and G3b) and albuminuria is categorized in 3 levels (A1-A3).
Image taken from: KDIGO 2012 Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease. *KI suppl. 2013; 3:1-150.* Published with permission from KDIGO.[10]

2.2 EPIDEMIOLOGY OF CKD

2.2.1 Causes of CKD in children

The main causes of CKD in adults are hypertension and diabetes, constituting two-thirds of all cases. In children, the etiology is very different from that in adults. The pediatric nephrology registry from North America (NAPRTCS) includes more than 7000 children and adolescents registered between 1994 and 2008. According to this registry, 58% of pediatric CKD cases are due to congenital causes, divided into congenital abnormalities of the kidney and urinary tract (CAKUT: 48%) and hereditary nephropathies (10%). Glomerulonephritis accounted for 14%, cystic kidney disease 5% and Hemolytic Uremic Syndrome (HUS) together with ischemic renal failure composed 4%.[11] In two large European registries from Italy and Belgium, similar distributions are reported[12 13].

Regarding the cause of CKD in pediatric patients with RRT, the same trends are observed in the ESPN/ERA-EDTA registry[3]. This is a European registry formed in 2007 which includes 37 countries and children with RRT aged 0-14 years. For nine of the countries, data on ages 0-19 years are also available. In their latest report, CAKUT was the dominant cause (41%) of pediatric RRT in Europe. The second largest cause was glomerulonephritis (15%), followed by cystic kidney disease (10%) and hereditary nephropathies (7%)[3]. Sweden has a similar

distribution of RRT etiologies. In a national survey of Swedish children in 1986-1994, 118 children were identified with GFR <30 ml/min/1.73m^2 [14]. The dominant cause of CKD was CAKUT (41%), followed by hereditary nephropathies (including juvenile nephronophthisis, autosomal polycystic kidney disease and congenital nephrotic syndrome: 26.5%), glomerulonephritis (14.5%) and vascular disorders (including HUS and ischemic renal failure: 10%)[14].

2.2.2 Demographics of CKD in children

There is limited information on the epidemiology of the early stages of CKD in the pediatric population, as it is often asymptomatic and therefore under-diagnosed and under-reported.

The prevalence of CKD (defined as GFR ≤75ml/min/1.73m² in the Italian registry or CKD stage 3-5 in the Belgian registry) ranged from 56-74.7 per million of the age-related population (pmarp), while the incidence was 11.9-12.1 pmarp[12 13]. Mean GFR at presentation was 42 ml/min/1.73m², supporting the fact that early CKD is under-diagnosed.

2.2.3 Demographics of RRT in children

Despite a stable incidence of CKD[1 15], the number of prevalent pediatric patients with RRT has increased in both Europe and the U.S., but now appears to have levelled off. In a European study including 12 countries with children aged 0-19 years, the prevalence increased from 22.9 pmarp in 1980 to 62 pmarp in 2000[15], mainly due to the increasing number of patients with a functioning kidney transplant. In the last decade the prevalence has been quite stable (58 pmarp in year 2011)[3]. A similar pattern is seen in the U.S., where the number of prevalent patients increased between 1996 and 2006, but has thereafter remained stable[1], Figure 3.

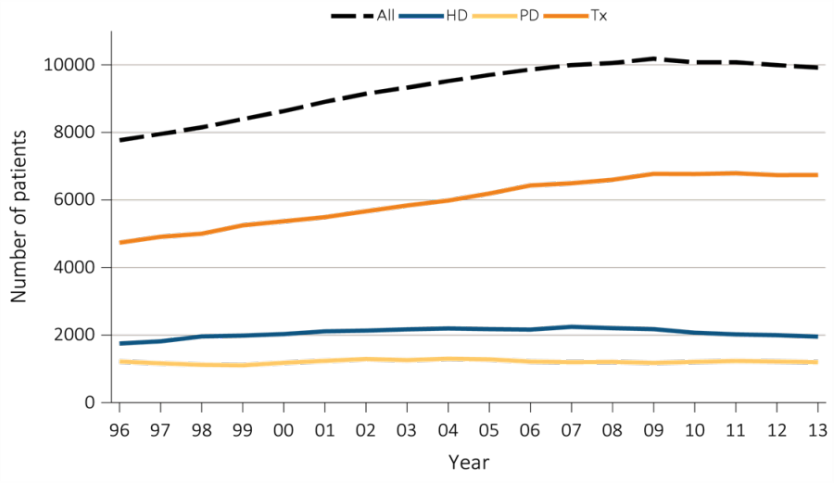


Figure 3. Prevalence of pediatric (0-21 years) renal replacement therapy (RRT) in the U.S. between 1996 and 2013. HD = hemodialysis; PD = peritoneal dialysis; Tx =renal transplant.
Image taken from: United States Renal Data System. 2015 USRDS annual data report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Betsheda, MD. 2015.[1] Published with permission.

Within Europe, the most recent report from the ESPN/ERA-EDTA registry shows considerable variation in RRT care[3]. Overall, among prevalent cases in Europe aged 0-14 years (27.9 pmarp), 74.6% were living with a renal graft (19.4 pmarp), 23.1% were on peritoneal dialysis (PD: 6.0 pmarp) and 13.8% were treated with hemodialysis (HD: 3.6 pmarp)[3]. However, when analyzing Swedish data separately, the prevalence of pediatric patients of the same age treated with a renal transplant was higher; 41.3 cases pmarp, while the prevalence for PD and HD was similar; 5.1 pmarp respectively[3]. In total, 81 patients aged 0-14 years were treated with RRT in Sweden at the end of year 2011[3].

Similarly, the incidence of RRT is higher in Western than in Eastern Europe, and also higher in Northern than in Southern European countries[16], Figure 4. This difference is thought to mainly reflect the macroeconomics of the country, with higher incidence of RRT in wealthy countries, but also due to differences in ethnic origin[16]. The incidence for the entire European cohort between 2009 to 2011 was 5.5 pmarp for ages 0-14 years[3], being approximately 20-fold lower than that of adults[17], while the incidence of RRT in Swedish children was among the highest at 10.8 pmarp[3]. RRT was started at a median GFR of 9 ml/min/1.73m² [3].

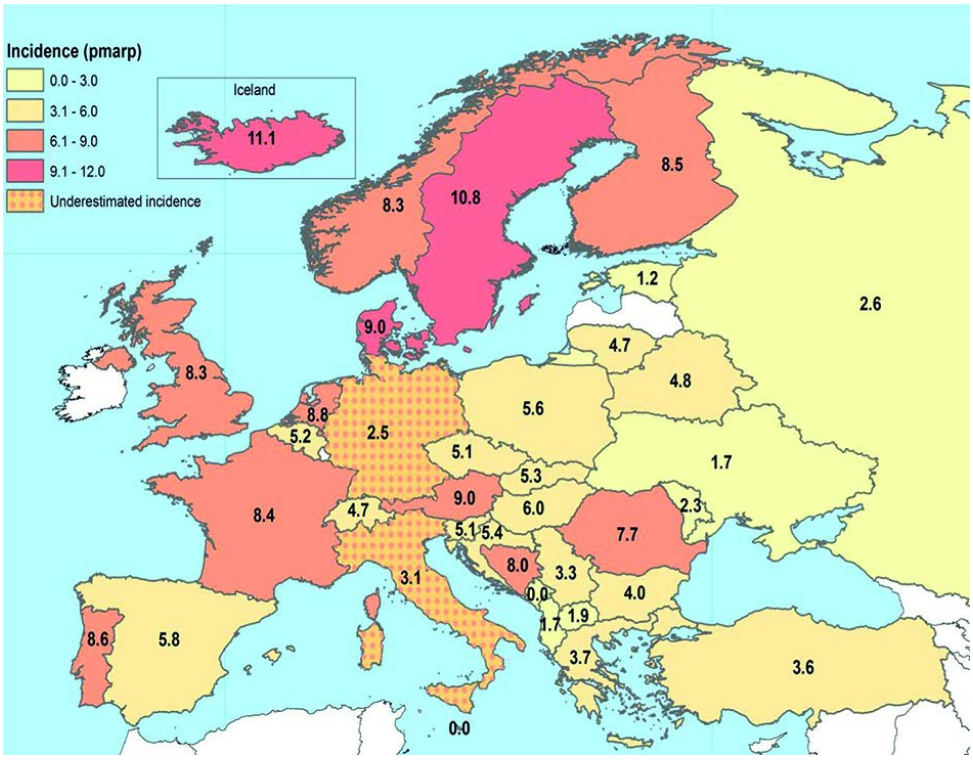


Figure 4. Incidence of pediatric (0-14 years) renal replacement therapy (RRT) per European country in 2009-2011. Pmarp=Per million age-related population. Data for Germany are based on transplantation patients only, and data from Italy does not include transplantation patients; consequently the values for these two countries are an underestimation.

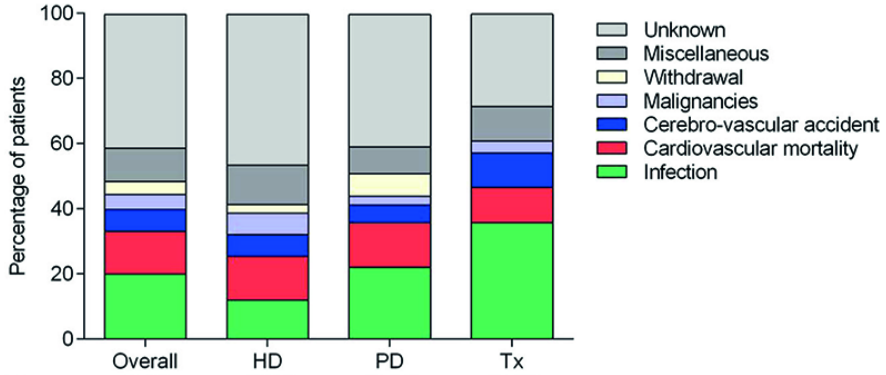
Image taken from: Chesnaye N, Bonthuis M, Schaefer F, et al. Demographics of paediatric renal replacement therapy in Europe: a report of the ESPN/ERA-EDTA registry. *Pediatr Nephrol 2014;29(12):2403-10*. Published with permission of Springer.[3]

2.2.4 Pediatric nephrology care in Sweden

Today there are four specialized pediatric nephrology centers in Sweden (Lund, Gothenburg, Uppsala and Stockholm). The majority of pediatric dialysis and renal transplant care is concentrated in these locations. Approximately 60% of all renal transplants in children in Sweden are performed at the Karolinska University Hospital Huddinge in Stockholm.

2.2.5 Mortality and morbidity in pediatric RRT

The four-year survival in children receiving RRT in Europe is 93.7% (PD 92.5%, HD 92.3% and renal transplant 99.1%). Patients starting RRT with dialysis have a 6.6 fold increased risk of death compared to those receiving pre-emptive transplantation[3]. However, across all European countries, only 19.6% of patients aged 0-19 years receive a pre-emptive renal transplantation, while 47.1% start with PD and 33% with HD[3]. The crude mortality rate for children with RRT was 20 deaths per 1000 patient years in ages 0-19 years and 23 deaths per 1000 patient years in ages 0-14 years[3]. This rate is 55-fold higher than that of the general population (0.42 deaths per 1000 children of the same age in Europe 2011)[18]. The cause of death in children and adolescents on RRT was unknown in 40.9% of cases. Infection and cardio/cerebrovascular events were equally important causes of death (19.9% each)[3]. In transplanted patients separately, 28.6% of all deaths were idiopathic, while infections accounted for 35.7% and cardio/cerebrovascular events 24.1% of all deaths, Figure 5. As expected from these data, the disease burden of CVD and infections is high in this population, with a hospitalization rate for CVD at 42 admissions per 1000 patient years and as high as 276 admissions per 1000 patient years for infections related complications[1].



	Overall	HD	PD	Tx
Unknown	40.9%	46.7%	39.7%	28.6%
Miscellaneous	10.2%	12.0%	8.2%	10.7%
Withdrawal	4.0%	2.7%	6.9%	0.0%
Malignancies	4.6%	7.7%	2.7%	3.6%
Cerebro-vascular accident	6.8%	7.7%	5.5%	10.7%
Cardiovascular mortality	13.1%	13.3%	13.7%	10.7%
Infection	19.9%	12.0%	21.9%	35.7%

Figure 5. Causes of death for pediatric patients aged 0-19 years starting renal replacement therapy (RRT) in 2009-2011. HD=Hemodialysis, PD=Peritoneal dialysis and Tx=Renal transplant.
Image taken from: Chesnaye N, Bonthuis M, Schaefer F, Groothoff JW, Verrina E, Heaf JG, et al. Demographics of paediatric renal replacement therapy in Europe: a report of the ESPN/ERA-EDTA registry. *Pediatr Nephrol 2014;29(12):2403-10.* Published with permission of Springer.[3]

Interestingly, when studying long-term survival, CVD seem to dominate in cause of death. In a report from the U.S. Renal Data Registry (USRDS)[19], 14.3% of patients receiving their first kidney transplant before age 21 (between 1983 and 2006) died over a median follow-up time of nine years. The majority of deaths were due to CV causes (34.6%) while infection was the cause of mortality in 19.5%. However, death rates due to CVD decreased for every year following renal transplantation, while deaths due to infections were stable over time[19]. Thus, while bearing in mind that this follow-up was longer, a shift in causes of death has occurred in the last decade. This may be a result of longer transplant survival. However, despite this possible improvement in CV mortality, the death rates are still approximately ten times higher in pediatric renal transplant recipients than in the general population[19]. Furthermore, among young adults who started RRT as children between 1985 and 2004, the average life expectancy was only 63 years for those with a functioning renal graft and as low as 38 years for those remaining on dialysis[20].

2.3 EPIDEMIOLOGY OF CVD IN CKD

In adults with RRT, arrhythmias and cardiac arrest was the most common cause of mortality, constituting 37% of all deaths according to the last report from USRDS[1]. Atherosclerotic heart disease composed the majority of all CVD in patients over the age of 44 years, while in patient aged 22-44 years, congestive heart failure was the most common cause of CVD. In patients aged 0-21 years, congestive heart failure constituted 19% of all CVD, followed by peripheral arterial disease (17%). In contrast to the aged population with RRT, this young cohort rarely demonstrated atherosclerotic heart disease (<4%).[1]

In a large population based cohort study, Go et al. examined more than 1.1 million adults, and identified a gradual increase in hazard ratios for adverse CV events as renal function decreased. The adjusted risk was 43% higher in those with GFR 45-59 ml/min/1.73m² and 343% higher in those with GFR <15 ml/min/1.73m² [21]. Similar data for a pediatric CKD population has yet to be published. Still, based on present published data, the American Heart Association´s guidelines for CV risk reduction in high-risk patients, place pediatric CKD in the highest risk category[22]. This group signifies a real risk of pathologic and/or clinical manifestation of CVD before age 30.

2.4 PREDICTION OF CARDIOVASCULAR RISK IN PEDIATRIC CKD

2.4.1 Risk factors and risk markers

The difference between risk factors and risk markers for a disease is not consistent through the literature. Overall, risk factors are thought of as being biologically causal in the path of a disease, and having measurable characteristics that precede and predict well-defined outcomes. Risk markers on the other hand, do not have to be causal factors. Rather, risk markers are biological indicators as a disease develops. The risk marker becomes a risk factor when it turns into a causal factor. Even though the causality of the following CV risk factors in CKD listed below is not fully established, they are often classified as just traditional or non-traditional CV risk factors. Non-traditional risk factors are, in the CKD population, often

referred to as uremia-related risk factors. Table 1 lists the most common risk factors in this context, which are included in this thesis.

Table 1. Traditional and uremia related cardiovascular (CV) risk factors[23].

Traditional CV risk factors:	**Uremia related CV risk factors:**
- Hypertension	- Anemia
- Obesity	- Chronic inflammation
- Dyslipidemia	- Albuminuria or Proteinuria
- Abnormal Glucose metabolism	- Altered mineral metabolism: Elevated Calcium-Phosphorus product, PTH and Vitamin-D deficiency

2.4.2 Traditional risk factors in pediatric CKD

In 2011, the Chronic Kidney Disease in Children (CKiD) study, an observational cohort study of 586 children aged 1-16 years with CKD stages 2-4, published comprehensive data on CV risk factors. Overall, 39% of participants had at least one risk factor, 22% had two risk factors and 13% had three present risk factors[24]. Patients with glomerular disease and nephrotic range proteinuria (in this publication defined as urinary protein to creatinine ratio above 2 g/g) had the highest odds of having several traditional risk factors present[24]. The number of prevalent risk factors increases as CKD progresses, and is highest in children on maintenance dialysis. Following kidney transplantation the prevalence of these traditional risk factors remains high. However, Kaidar et al. recently showed that in 77 renal transplant patients the number of risk factors present decreased progressively following renal transplantation to the last follow-up visit (on average seven years).[25]

2.4.2.1 Hypertension

Hypertension in pediatric CKD results from volume expansion and increased vascular resistance, which develops as renal function deteriorates. In children, uncontrolled hypertension is defined as a blood pressure $\geq 95^{th}$ percentile for that child´s age, gender and height. Controlled hypertension is defined as the need of antihypertensive medications in order to regulate blood pressure levels to $<95^{th}$ percentile. Hypertension is considered a traditional risk factor for CVD, important in several patient groups as well as the general pediatric population[26 27]. In children with CKD, hypertension is associated with deterioration in renal, cardiac and vascular functions[28-32]. Furthermore, in adults the use of angiotensin-converting enzyme inhibitors (ACE-I) and/or angiotensin-II receptor blockers (ARB) to treat hypertension in CKD reduces the risk of kidney failure, CV death and mortality by all other causes[33].

CKD: In data from the CKiD cohort, 21% were normotensive, 37% had elevated blood pressure and 42% had controlled hypertension[24 34]. In the same cohort, 14% and had uncontrolled systolic hypertension and 13% uncontrolled diastolic hypertension using office

blood pressures[35]. High BMI and elevated levels of proteinuria were important risk factors for a longitudinally increasing blood pressure[34]. Equally, ambulatory blood pressure (ABP) measurements revealed that 19% had masked hypertension (elevated ambulatory blood pressure, but normal office blood pressure) and 13% confirmed hypertension (elevated office and ambulatory blood pressure)[35]. Further, a high variability in mean systolic ABP was seen, but also in night diastolic ABP in hypertensive children[36].

CKD-T: The prevalence of hypertension increased in the immediate and short term following renal transplantation and was 52% two months after transplantation, and decreased to 27.5% after six months, and 22% two years after transplantation[25]. At the same time points, 54.8%, 38% and 37.7% of patients were treated with antihypertensive medication[25]. In another study, 27.9% of children were normotensive six months after renal transplantation and not treated with antihypertensive drugs. Of these non-hypertensive patients post-transplant, 49.3% became hypertensive and commenced antihypertensive medication during the follow-up of two years[37]. Still, long-term prevalence of uncontrolled hypertension 7-18 years after renal transplantation was only 12%-14%[38 25].

2.4.2.2 Dyslipidemia

Dyslipidemia in CKD is characterized by increased levels of plasma triglycerides (TG) and triglyceride-rich lipoproteins [very low density lipoprotein cholesterol (VLDL) and Chylomicron remnants], as well as decreased high density lipoprotein cholesterol (HDL) and apolipoprotein A1. Chylomicron remnants and VLDL accumulate in CKD patients due to increased production and impaired catabolism[39]. The KDIGO recently published guidelines with cut-offs for acceptable, borderline high and high levels for cholesterol, LDL and non-LDL in children[40].

The potential impact of dyslipidemia on CVD in the general population is profound. Indeed, elevated lipid levels in children without renal disease present a risk factor for later CVD[41 42]. However, the relative risk of CVD from dyslipidemia in children with CKD compared to the general pediatric population is not known. In adult CKD patients, including those with RRT, the definite role of dyslipidemia in the development of CVD is debated and remains unclear.

CKD: The prevalence of dyslipidemia in children with CKD is high, and is dependent on the cause and duration of CKD. Dyslipidemia is more common and severe in patients with glomerular disease and proteinuria, as well as in late stage CKD[43 44]. Specifically, elevated levels of triglycerides and non-HDL, as well as the use of lipid lowering drugs, have been reported in 44% of children with CKD stages 2-4[24]. As GFR declines, both triglyceride and cholesterol levels increase[44].

CKD-T: In pediatric renal transplant patients the prevalence of hypercholesterolemia and triglyceridemia remains high[45], and was reported to reach 49% and 50%, two months after transplantation. After seven years the prevalence was 33% and 13% respectively[25]. The major cause of dyslipidemia in this patient group is not only progressive loss in renal function, but

can also be attributed to medications used; particularly corticosteroids, cyclosporine and sirolimus[46].

2.4.2.3 Abnormal Glucose metabolism

In CKD, glucose intolerance is primarily a result of impaired tissue sensitivity to insulin with several possible causes discussed in the literature[47 48]. Glucose intolerance involves impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes mellitus. The American Diabetes Association (ADA) and WHO use different definitions of these abnormalities, which generates confusion when comparing studies. The cut-off for glucose to define IFG ranges from 5.6 (ADA) to 6.1 (WHO) mmol/L[49 50]. The definition for IGT is an oral glucose tolerance test (OGTT) with 2 hour glucose level of 7.8 to 11.0 mmol/L[49 50]. Moreover, while fasting insulin levels identifies those with hyperinsulinemia, the homeostasis model assessment index for insulin resistance (HOMA-IR) is used to assess insulin resistance[51]. While other measures of insulin resistance (intravenous glucose tolerance test and glucose clamp technique) are more robust, they are invasive and usually not possible to perform in routine clinical check-ups.

Alterations in glucose and insulin metabolism play an important role in the development of CVD in other populations and might also be of importance in adult CKD and CKD-T patients. For example, "new-onset diabetes after kidney transplantation" (NODAT) is associated with impaired renal survival and an increased CV morbidity and mortality in adult patients[52-55]. Very few data are available for the impact of abnormal glucose metabolism in pediatric CKD.

CKD: Although diabetes is a rare cause of CKD in children, IFG and IGT are common in pediatric CKD, including dialysis patients. Using ADA´s definitions, 35% of pediatric non-dialysis CKD patients exhibit either IFG or IGT[56]. While the prevalence for hyperinsulinemia varies between studies of pediatric CKD stage 2-4 (9%-33%), insulin resistance reveals similar prevalence (16%-19%)[24 57]. Moreover, using the WHO´s definition for IFG, overall 21% of pediatric patients in the CKiD cohort had abnormal glucose metabolism defined as IFG, hyperinsulinemia or insulin resistance[24].

CKD-T: Glucose intolerance is also common following kidney transplantation in children (10%-16%)[25 58]. NODAT also exists, but is not as common as IFG or IGT. In a study from the NAPRTCS registry, 3% of pediatric patients developed a need for insulin treatment following renal transplantation[59], while other studies have reported a prevalence of NODAT as high as 7%-13%[60 58]. Greenspan et al. showed that the incidence of NODAT increased with time from 2.1% in 1986-1990 reaching 20% in 1996-1999. This increase occurred in the same era as tacrolimus was introduced, and today it is clear that many immunosuppressant agents used in CKD-T patients (corticosteroids, calcineurin inhibitors and sirolimus) affect carbohydrate metabolism negatively. For example, the insulin resistance is increased and insulin secretion is decreased[61]. However, also of importance is the simultaneous increasing incidence of both Type I and II Diabetes in children worldwide[62].

The high prevalence of these traditional risk factors; hypertension, dyslipidemia and abnormal glucose metabolism, cannot fully explain the increased rates of cardiac death in adults or children with CKD and RRT. In this context uremia-related risk factors have generated much interest, which will be presented next.

2.4.2.4 Anemia

Anemia in CKD is predominantly caused by erythropoietin deficiency, but other factors such as acidosis, inflammation and malnutrition related to uremia contribute as well[63]. Due to various definitions of anemia used historically[64-66], comparisons between studies have sometimes been troublesome. Following guidelines recently presented by KDIGO with cut-offs to define anemia in children at different ages, this issue is hopefully transient[67].

Anemia is a well-known risk factor for CVD in adult CKD[68], with an increased incidence of hospitalization as compared to non-anemic pediatric CKD patients[66]. Also, anemia in children with chronic dialysis has been associated with overall mortality, but not to cardiac-related death[69]. Likewise, in pediatric CKD-T patients lower hemoglobin was associated with increased risk of death, and also graft loss[70].

CKD: Despite the use of erythropoiesis-stimulating agents and iron therapy, anemia is present during early stages of CKD and is often poorly controlled, especially in children with advanced CKD[35]. Indeed, in a large study of pediatric CKD, the prevalence of anemia was found to range from 18.5% in CKD stage 2 to 68% in CKD stage 5[66].

CKD-T: Anemia sometimes remains after renal transplantation. Thus, 30% of pediatric patients at two months after transplantation and 18% after seven years are anemic[25]. In a very recent large multicenter study, the prevalence of anemia, ranged from 7.8% to 49.8% depending on the cut-off used[70]. When the definitions of anemia encompassed erythropoietin treatment, the prevalence increased to 16.3% and 58.1%. Hemoglobin levels were associated with pre-transplant care (pre-emptive transplantation vs. previous dialysis treatment), graft function and antihypertensive and immunosuppressive medications[70].

2.4.2.5 Chronic inflammation

A variety of factors contribute to chronic inflammation seen in patients with CKD and RRT, including increased production and reduced clearance of pro-inflammatory cytokines, oxidative stress and acidosis as well as chronic and recurrent infections. There is an inverse correlation between GFR and level of inflammatory cytokines as well as a positive correlation between albuminuria and inflammation[71]. The relationship between inflammation and CVD in the general population is well known and chronic inflammation is also a well-established risk factor for morbidity and mortality in adult dialysis patients[72]. Different biomarkers of inflammation appear to have varying predictive values. For example, Interleukin-6 (IL-6) predicts all-cause and CV mortality more accurately than C-reactive protein (CRP) and other cytokines[73 74]. The postulated mechanism of inflammation in CVD is that chronic inflammation promotes vascular calcification and endothelial dysfunction[75]. Also

in adult non-dialysis CKD, inflammation may play an important role in the development of cardiac morbidity[76].

CKD: While chronic inflammation is present in pediatric CKD and dialysis patients[77-79] , its role in this group remains conflicting[79-81].

CKD-T: Inflammation is also discussed as a potential CV risk factor in pediatric CKD-T patients, but very few studies are available [82]. The significance of inflammation in this group, receiving a variety of immunosuppressive agents is difficult to interpret.

2.4.2.6 Protein-Energy Wasting (PEW)

Inflammation has a role in the development of protein-energy wasting (PEW)[83], which is common in patients with chronic dialysis treatment. PEW represents a condition with decreased body stores of protein and fat mass[84], and is together with chronic inflammation, associated with increased risk of morbidity and mortality in adult patients[85]. Hypoalbuminemia, as a marker of PEW, is also associated with increased mortality in pediatric dialysis patients[86].

2.4.2.7 Albuminuria

Small proteins such as albumin may pass the glomerular filtration barrier, but usually they are reabsorbed in the proximal tubule. Albuminuria refers to excessive loss of albumin in the urine due to abnormalities either in the glomerular barrier or in the tubular reabsorption system. The presence of persistent albuminuria is an early sign of renal damage and is closely related to the progression of CKD in children[87]. As for several other CV risk factors mentioned previously, comparing studies on albuminuria and proteinuria is troublesome as they often use different definitions. Microalbuminuria is often defined as spot sample urinary albumin of 30-300 mg/L and above this limit is macro-albuminuria. The KDIGO 2012 guidelines refers to albuminuria as normal or mildly elevated if urinary albumin to creatinine ratio is <30 mg/g (3 mg/mmol), moderately increased if 30-300 mg/g (3-30 mg/mmol) and severely increased if above 300 mg/g (30 mg/mmol), Figure 2.

In addition to its role as a marker for CKD risk, it is now widely accepted that albuminuria is an independent predictor of CV morbidity and mortality across various populations[88]. The pathophysiological link between albuminuria and CVD has been suggested to be related to, in particular, endothelial dysfunction[89].

CKD: Albuminuria is common in pediatric CKD and in the CKiD cohort, 71% had an elevated urinary protein to creatinine ratio. About 20% of these patients had nephrotic range proteinuria[34]. Longitudinal pediatric studies have shown that albuminuria is an independent predictor for CKD progression and increasing blood pressures[34 90].

CKD-T: Following renal transplantation the rate of albuminuria usually falls, and persistence or late appearance of albuminuria represents graft injury with the mechanism being

multifactorial[91]. In a small study of 53 renal transplanted children, 47% had pathologic urinary protein to creatinine ratio[92].

2.4.2.8 Abnormal mineral metabolism

In mineral metabolism there is a complex interaction between the kidneys, the parathyroid gland, the gastrointestinal tract, and the skeleton. In children with CKD, these mechanisms for maintaining normal serum calcium, phosphorus, and 1.25-dihydroxyvitamin D (1.25-vitamin D) concentrations are disrupted. In 2006, the working group for KDIGO proposed that the mineral and bone disorder in CKD patients should be referred to as CKD-MBD[93]. CKD-MBD includes systemic disturbances that may be present alone or which may be a combination of the following three conditions: (1) laboratory abnormalities of calcium, phosphorus, PTH or vitamin D; (2) bone abnormalities in turnover, mineralization, volume, linear growth or strength and (3) calcification of the vasculature or other soft tissues[93].

Phosphorus retention begins during the earliest stages of CKD and plays a central role in the development of hyperparathyroidism. Ongoing research has identified other factors of importance in the regulation of phosphorus balance; Fibroblast growth factor 23 (FGF23) and Klotho. FGF23 is produced by bone cells (osteocytes and osteoblasts) and circulates in blood to bind to its receptor FGFR and co-receptor Klotho in the kidney. Figure 6 shows the complex PTH - vitamin D - FGF23 axis and actions in different organs.

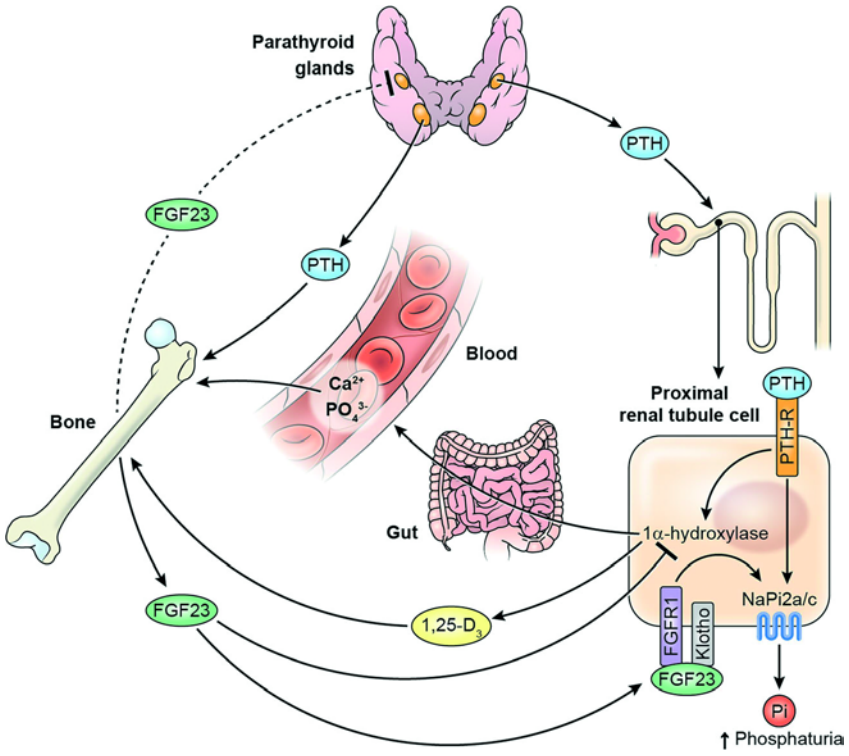


Figure 6. The Parathyroid Hormone (PTH) - Vitamin D – Fibroblast Growth Factor 23 (FGF23) axis showing interaction between parathyroid glands, bone, kidney, and gut.
Image taken from: Blau JE, Collins MT. *Rev Endocr Metab Disord (2015) 16: 165-174.* Published with permission from Springer.[94]

FGF23 together with Klotho reduce the expression of sodium phosphate co-transporters (NaPi2a/c) in the proximal renal tubule in the kidney to induce phosphaturia, similar to PTH. However, in contrast to the action of PTH to induce 1α-hydroxylase to convert 25-hydroxyvitamin D (25-vitamin D) to 1.25-vitamin D, FGF23 acts as a counter-regulatory hormone of phosphate balance and consequently suppress 1α-hydroxylase. The ensuing low levels of 1.25-vitamin D leads to decreased calcium and phosphate absorption from the gut and depressed bone mineralization. In more advanced stages of CKD, this adaptation becomes less successful, and as a result hyperphosphatemia is more commonly found.[94]

The role of CKD-MBD on mortality is not fully clear. Low levels of 25-vitamin D are associated with all-cause and CV mortality in adult hemodialysis patients[95 96], and are associated with worsened cardiac morbidity and progressive renal failure in pediatric CKD[97 98]. However, recent meta-analyses have not been able to prove that vitamin D supplementation affect mortality or CV risk in adult CKD patients, with or without RRT[99 100]. In addition, FGF23 levels increase in patients with vitamin D supplementation and high FGF23 are associated with excessive morbidity and mortality in adults with RRT[95 101-104] . Further, while hyperphosphatemia also has been independently associated with mortality in adult CKD[105], it is clear that FGF23 increases as CKD progresses, and becomes maladaptive and possibly contributes to cardiac remodeling independent of phosphate levels[106-108]. In addition, while FGF23 increase, Klotho decrease as the renal function deteriorates[109]. Low levels of soluble Klotho has been found to be involved in vascular calcification in CKD and other populations, but has not been linked to increased mortality[109-111]. However, the numbers of studies are few.

CKD: Abnormal mineral metabolism is common in children with CKD and becomes more prevalent as kidney function decreases. Although children with early stage CKD generally have no signs or symptoms of bone abnormalities, laboratory testing may already show decreased 25-vitamin D and elevated PTH[112 113]. Current treatments focus on suppression of PTH with vitamin D supplementations. Subtle signs of bone osteodystrophy may begin in CKD stage 3 with muscle pain, weakness and bone deformations. In a small report of pediatric CKD, bone biopsy demonstrated increased bone turnover in 0%, 13% and 29% and defective mineralization in 29%, 42% and 79% in CKD stage 2, 3 and 4/5 (before dialysis) respectively[114]. FGF23 levels increase as CKD progresses from stage 1 to 5 in children with the most marked elevation in advanced CKD[115-119]. Serum FGF23 was increased in 67% of children with CKD stage 2-4 and was found to be the earliest detectible abnormality in mineral metabolism[115]. This has also been shown in adult CKD[120 121]. In addition, serum levels of soluble Klotho are reduced in children with CKD[116].

CKD-T: FGF23 is elevated also in CKD-T children[116 122 123], in which hyperparathyroidism is also common (32% two months after transplantation and 18% after seven years)[25]. Levels of soluble Klotho in pediatric CKD-T have only been published in very few previous studies[116 124].

2.5 THE HEART

The heart wall consists of; endocardium (endothelial cells and loose connective tissue), myocardium (cardiomyocytes and connective tissue), epicardium (loose connective tissue and epithelium) and pericardium (inner serous and outer fibrous structures protecting the heart), Figure 7.

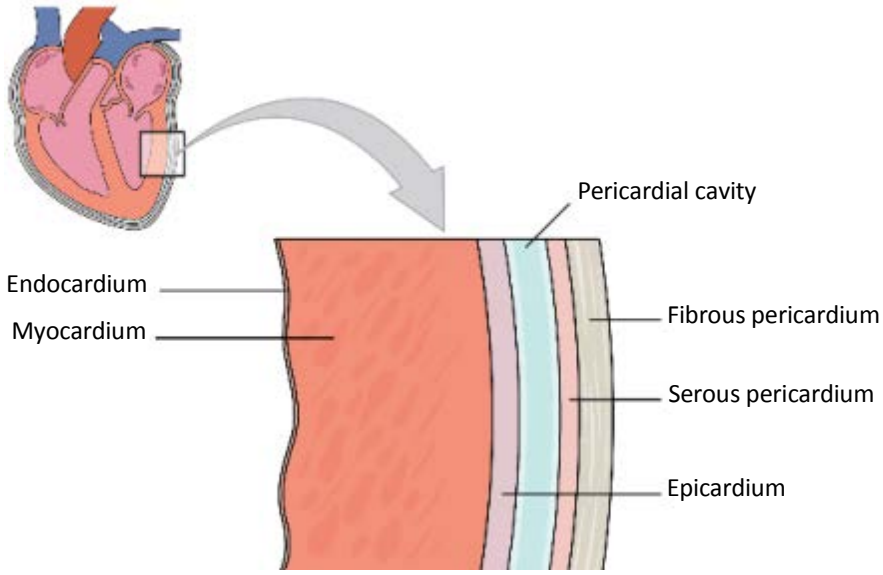


Figure 7. Layers of the heart wall.
Image taken from*: https://creativecommons.org/licenses/by/3.0/deed.en.* This file is licensed under the Creative Commons Attribution 3.0 Unported license.

The cardiomyocytes are small cells dependent on aerobic metabolism to obtain energy for contracting, making them vulnerable to decreased oxygen supply. Collagen and elastic fibers surround the myocytes to provide support and strength.

2.5.1 Uremic Cardiomyopathy and Cardio-Renal Syndrome

While the mechanisms leading to the increased risks for CV complications among CKD patients are not fully understood, there is growing awareness of the interdependence between kidney and heart. The kidneys affect the heart function by regulating body salt and water content. The kidneys in turn depend on blood flow and pressure generated by the heart. In the 1980s, echocardiographic studies identified adverse changes in cardiac structure and function associated with chronic dialysis treatment that were termed uremic cardiomyopathy (UC)[125]. In the most recent literature, a new concept describing the close relationship between kidneys and heart has emerged as “Cardio-Renal Syndrome” (CRS). CRS was proposed to be a condition characterized by declining function in both kidneys and heart, where failing function of one organ worsens the function of the other, thus further accelerating the progressive failure of both organs[126]. Clinical classification of CRS is based on the type and extent of organ dysfunction. Currently, five types have been established and CRS type 4

represents a disease state where CKD is a causal factor in the development of cardiac dysfunction.

2.5.2 Cardiac remodeling and LVH

Cardiac remodeling refers to a state of changed size, shape, structure and physiology of the heart. The cardiac myocyte is the major cell involved in remodeling, which occurs as a physiological response to an increased demand or due to injury of the myocardium. The pathophysiological factors involved are divided into three categories: (1) related to afterload, (2) related to preload, and (3) not related to afterload or preload. Factors affecting afterload include elevated arterial blood pressure and reduced large-vessel compliance[127]. Factors affecting preload are intravascular volume expansion (salt and fluid loading) and secondary anemia[128 129]. These related factors often have compounding and synergistic effects.

Cardiac remodeling is usually assessed using echocardiography to estimate left ventricular mass (LVM). Measurements of the left ventricular internal dimensions and the thickness of the posterior and myocardial septal walls defined by the endo- and epicardial borders are included in the assessment of LVM. The relative wall thickness (RWT) can also be assessed using the same measures. The LVM-index (LVMI) adds an adjustment for body size. A pathologically increased LVMI is referred to as left ventricular hypertrophy (LVH), which can further be classified as concentric or eccentric.

Concentric hypertrophy (or symmetric hypertrophy) is caused by factors related to increased afterload, leading to disproportionate thickening of cardiomyocytes with enlarged intraventricular septum and left ventricular posterior wall, while the LV internal diameter remains unchanged. This remodeling increases both the relative wall thickness (RWT) and left ventricular mass index (LVMI) and enables the left ventricle to generate greater forces and higher pressures. Eccentric hypertrophy (or asymmetric hypertrophy) on the other hand is a result of increased preload causing cardiomyocyte lengthening which leads to a dilation of the LV internal diameter, enabling the LV to expand more in order to receive greater volume of blood. The wall thickness normally increases in proportion to the dilation of the LV, with a predominant thickening of the intraventricular septum. So, while LVMI is increased, RWT is normal.

2.5.2.1 Important considerations regarding LVMI and LVH

The variability in echocardiographic measurements assessing LVH is high in both adult and pediatric populations. For example, the prevalence of LVH across studies of pediatric CKD varies from 17%-49%[6 130 80 131 132]. In part, this can be explained by variations in the populations examined, but the prevalence of LVH also varies within CKD stages in children; ranging from 11%-31% in CKD stage 3 and 7%-40% in CKD stage 4[133 134]. So other factors, like differences in the indexing method chosen to assess LVMI, as well as reference population used to estimate LVMI percentiles or LVM z-scores and the threshold value chosen to define LVH also seem to be of importance[80 132 135 136]. Also variations between observers when measuring LVM have been acknowledged previously[137].

2.5.2.2 Normative Data for LVM in children

Foster et al. recently published normative data (n=440) from birth to age 21 years, allowing calculations of LVM z-scores related to height percentiles[135]. Additional reference material was published by Khoury et al. (n=2273) in children and adolescents aged 0-18 years allowing assessments of LVMI percentiles related to age[136]. Both publications proposed a cut-off for LVH at the 95th percentile.

2.5.2.3 LVH in CKD

CKD: As previously mentioned the prevalence of LVH varies (17%-49%) across studies in pediatric CKD[6 80 130-132]. Also regarding the prevalence of concentric or eccentric hypertrophy, the information diverges. For example, a recent study of children with stage 2-5 CKD revealed that the overall prevalence of LVH was 38%, but eccentric hypertrophy only existed in patients with GFR <15 ml/min/1.73m², while concentric hypertrophy was more common even in milder disease states[134]. In contrast, another study of pediatric patients with CKD stage 3-5 (non-dialysis) showed that 36% had LVH, all being eccentric hypertrophy and none concentric[138]. Similarly, in a comparable pediatric cohort, LVH was present in 38% of all patients, again all eccentric[81].

CKD-T: Children with a renal transplant also show signs of cardiac remodeling, with the prevalence of LVH being 40% one year post-transplant[139]. Furthermore, six years after renal transplantation, the overall prevalence of LVH was 21%[140], with the majority being eccentric hypertrophy. Similarly, in a recent longitudinal cohort study following pediatric renal transplant recipients up to ten years after transplantation, the prevalence of LVH dropped from 33% to 0-25% depending on the definition used for LVH[141].

2.5.2.4 Cardiac remodeling and mortality

In adult patients starting RRT, LVH is associated with increased mortality rates[125 142], and is common across all CKD stages[143]. LVH is also a strong independent risk factor for cardiac arrhythmia, and heart failure in patients undergoing dialysis[144 145]. Importantly, in studies using LVH as a risk factor, a graded relationship between LVMI, being a continuous variable and CV risk is not evaluated.

2.5.3 Systolic and Diastolic Cardiac Function

The heart cycle is divided into systole (ejection phase) and diastole (filling phase). Systole is defined as the time between the closure of the mitral and aortic valve, while diastole is defined as the time between the aortic and mitral valve closure.

The movements in the ventricle walls are an interplay between the longitudinal fibers, situated for the most part subendocardially, and the radial fibers located in the midwall[146]. The position of the longitudinal fibers makes them vulnerable to disturbed perfusion, as the coronary arteries are located on the surface of the heart. Thus in cases of increased wall stress, caused by for example hypertension, the perfusion to the longitudinal fibers will

decrease, leading to disturbed function. The location of the mitral annulus makes it an ideal place to measure velocities in the longitudinal fibers. An initial function loss in the longitudinal fibers is compensated for by the radial fibers, thereby preserving ejection fraction (EF), i.e. the percentage of the end diastolic volume ejected during systole. It is not until the function of the radial fibers also fails that the EF is decreased.

The diastolic cardiac cycle can be divided into two phases; the fast filling phase (E) representing early diastole, and the final filling phase due to atrial contraction (A) representing late diastole. During the fast filling phase (E) the largest change in volume occurs, while atrial contraction (A) contributes to 15%-30% of the total volume in the LV. The filling of the ventricle is determined by the volume in the atrium, the residual volume in the ventricle and pressure gradients in atrium and ventricle. In simple terms, the diastolic function is determined by the filling volume and the compliance of the left ventricle. Using echocardiography the diastolic function can be assessed with conventional pulse wave Doppler (PWD) measuring blood flow velocity over the mitral valve, and/or tissue Doppler imaging (TDI) measuring myocardial velocities in the annulus mitralis[147].

2.5.3.1 Conventional Pulse Wave Doppler (PWD)

Traditional echocardiographic assessments of LV diastolic function rely on conventional pulse Wave Doppler (PWD) patterns of mitral inflow, Figure 8.

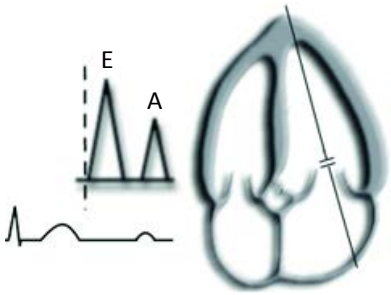


Figure 8. PWD sample volume is placed at the tips of the mitral valve in the left ventricle. Two flow velocities can be seen during diastole: the E-wave, representing the early, passive filling due to relaxation of the left ventricle, and the A-wave, that happens late in diastole, representing the active filling due to atrial contraction. Image taken from: *http://www.echobasics.de/diastole-en.html* with permission from the author Dr Merelez D.

Normally, the E-wave velocity is greater than that of the A-wave. Transmitral velocities are directly related to atrial pressure (preload) and inversely related to ventricular relaxation[148]. As LV function deteriorates, the atrial pressure rises in response to reduced LV compliance in order to maintain filling properties. This increase in pressure masks the influence of impaired relaxation and the mitral inflow velocity (PWD E) is thought to be normal to high instead of reduced[149]. In practice, the use of only mitral valve inflow patterns to assess diastolic dysfunction remains limited.

2.5.3.2 Tissue Doppler Imaging (TDI)

As early as 1989, Isaaz et al. were the first to realize the clinical and diagnostic potentials of tissue Doppler imaging (TDI)[150]. In the following years, other scientists improved the technique for producing images of velocity of tissue motion within the myocardium[151]. Already in 1994, Sutherland and Flemming et al. demonstrated the feasibility of color TDI to assess myocardial function[152] and since about year 2000, TDI has been used in clinical practice. This technique offers the ability to quantify regional and global LV function, both systolic velocities (s´) and early (e´) and late (a´) diastolic velocities, Figure 9.

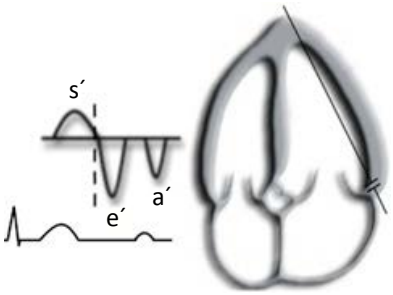


Figure 9. TDI sample volume is placed at the level of the lateral mitral annulus. The s´ peak represents the systolic myocardial velocity, while the e´ corresponds to early diastolic velocity and the a´ to late diastolic velocity peaks.
Image taken from: *http://www.echobasics.de/diastole-en.html* with permission from the author Dr Merelez D.

There is evidence that TDI is less influenced by changes in preload than PWD. In fact, myocardial velocities are persistently reduced even in those stages of diastolic dysfunction characterized by increased preload compensation[149]. In cases of increased afterload, both systolic and diastolic myocardial peak velocities are altered[149]. It is well established that correcting mitral inflow velocity (E) for the influence of LV myocardial relaxation (e´) using the ratio E/e´, enables a good estimate of LV filling pressure[153 154]. So, while a reduced e´ peak velocity recognizes patients with abnormal LV relaxation independent of atrial pressure, an elevated E/e´ ratio identifies those with increased filling pressures[153 154].

There are two tissue Doppler techniques: pulse wave (PW)-TDI and color coded (cc)-TDI, the latter being an extension of PW-TDI. Cc-TDI has advantages over PW-TDI with increased spatial resolution and the ability to perform analyses off-line as well as the possibility of evaluating multiple structures and segments in a single view. Also, cc-TDI allows other analyses such as speckle tracking and strain rate imaging. Although many clinics use cc-TDI in their daily work, PW-TDI is still the recommended technique since validation studies have been made using PW-TDI[147]. Nonetheless, studies have shown a high correlation between PW-TDI and cc-TDI[155]. Hummel et al. show in 114 adult patients that both PW-TDI and cc-TDI e´ ($r=0.93$, $p<0.001$) as well as E/e´ derived from PW-TDI and cc-TDI ($r=0.85$, $p<0.001$) are closely correlated. This study also shows higher e´ velocities and lower E/e´ values for PW-TDI than those derived from cc-TDI and presents converting formulas for e´

and E/e´. It is important to point out that when comparing data derived from TDI in different studies, small discrepancies may arise due to differences in sample placement as well as choice of sample size. Also, differences in transducer angulation and filters used to reduce noise can affect the results.

2.5.3.3 Normative data for cardiac function in children

Cantinotti et al. recently published a critical review of published nomograms for PWD and TDI[156]. From this review, it is clear that most published nomograms are based on small samples and there is a great heterogeneity in the methodologies used. For example, different variables are included in adjusted models and the normalized data are presented differently between studies (z-scores, percentiles and mean values). Indeed, although most studies adjusted measurements for age, different age groups were used and few adjusted for body size and heart rate, making comparisons difficult. The largest study by Eidem et al., presented normal reference values derived from 325 children aged 0 to 18 years adjusted for body size and heart rate[157]. Recently, data derived from cc-TDI were also published by Dallaire et al[158]. This study includes 233 healthy children aged 1-18 years and uses body surface area to adjust for differences in body composition. Regression equations allow for the calculation of z-scores for PWD (E, A and E/A) as well as cc-TDI indices (e´, a´).

2.5.3.4 Definitions for Left Ventricular diastolic dysfunction (LVDD)

When evaluating left ventricular diastolic dysfunction (LVDD), it is of interest to assess several estimates derived from the echocardiographic examination. The American Society of Echocardiography together with the European Association of Echocardiography have proposed a grading system when assessing LV diastolic function including estimates for e´ and left atrial (LA) volume, E/A and e´/a´ ratio, the E-waves deceleration time (DT) and E/e´ ratio[147 149]. Regarding e´ peak velocity, which is considered a reliable marker for impaired LV relaxation, less than -2 z-scores is pathologic and indicates possible LVDD[147]. Additionally, the cut-off for LVDD using E/e´ ratio (as assessed with PW-TDI) is above 15, while possible LVDD includes an E/e´ ratio of 8-15 and normal diastolic function is a ratio below 8. Using the proposed conversion formula published by Hummel et al., E/e´ cut-off values derived from cc-TDI for LVDD is above 20, and possible LVDD if 11-20 as well as normal if below 11[155]. Importantly, these cut-off values are based on a population aged 18 years or older. Eidem et al. showed that e´ increases while E and a´ velocity peaks are stable during childhood[157]. Thus, E/e´ and e´/a´ will both decrease with age in children. Despite this, the same cut-off values for E/e´ have been proposed for children above the age of three years, which might underestimate the true prevalence of LVDD in pediatric cohorts[156].

2.5.3.5 Left Ventricular Diastolic dysfunction (LVDD) in CKD

A close correlation between LVH and cardiac dysfunction is well described in CKD. Myocardial hypertrophy induces the activation of cellular apoptotic signals and activates metabolic pathways able to increase extracellular matrix production (fibrosis)[159 160], possibly leading to the subclinical systolic and diastolic dysfunctions detectable early in CKD

progression[161]. Recent studies have also shown signs of decreased cardiac function in CKD patients without LVH. In detail, LV function was closely correlated with elevated markers of diffuse interstitial myocardial fibrosis assessed by cardiac magnetic resonance (CMR) imaging[162]. Fibrosis leads to progressive impairment of contractility with stiffening of the myocardial wall causing both systolic and diastolic dysfunction. Importantly, it is not clear whether LV dysfunction arises as a result of the presence of interstitial fibrosis or whether both are consequences of damage in CKD that occurs as part of the final common pathway to uremic cardiomyopathy/CRS type 4[162].

CKD: Historically, diastolic function as assessed by conventional PWD shows that LV diastolic function is affected in children with CKD[6]. In more recent years, TDI has emerged as a more sensitive and reliable technique that can detect LVDD in the same population[134 163-167]. How LV diastolic function changes longitudinally in this patient group is not explored.

CKD-T: LVDD in pediatric CKD-T has only been assessed in a few small cross-sectional studies; using PWD[6] and in more recent years also TDI echocardiography, altogether revealing that cardiac dysfunction persists following renal transplantation[168-171].

Interestingly, these pediatric CKD and CKD-T studies have foremost reported data on E, A, E/A, e´, a´, e´/a´ and E/e´ as indicators of diastolic function. Data for indexed LA volume was only reported in two studies and was increased compared to controls[163 168], while E-wave DT was similar to controls in two reports[169 171].

2.5.3.6 Cardiac dysfunction and mortality

Using TDI in adult patients with CKD stage 4-5 (including dialysis) with a normal LV mass and preserved systolic function as measured by EF, Rahkit et al. demonstrated that at least one marker of either LV deformation (strain) or early myocardial relaxation (e´) velocity was reduced in all patients[7]. These changes independently predicted an increase in all-cause mortality and cardiac mortality over follow-up[7]. Along with systolic dysfunction, LVDD has emerged as a major risk factor for death and CV events in this patient group[172].

The prognostic impact of the TDI indices of systolic and diastolic function was recently assessed in the Copenhagen City Heart study, a large population-based study including 2064 subjects followed prospectively. While a reduced e´ velocity independently predicted acute myocardial infarction, both s´ and a´ velocity peaks were independent predictors of heart failure and CV death after adjustments of traditional risk factors and conventional echocardiography measures. TDI estimates remained important predictors for the combined end-point even in a subgroup with normal conventional echocardiography.[8]

2.6 THE ARTERIES

The arterial walls are composed of three layers; tunica intima, tunica media and tunica adventitia (externa). These three layers are separated by the internal elastic lamina between the intima and media, and the external elastic lamina separating the media and the adventitia. The inner surface of the tunica intima is fronted by endothelial cells. The tunica media in large arteries is mainly composed of elastic fibers, while in muscular arteries the vascular smooth muscle cells (VSMCs) dominates.

2.6.1 Carotid intima media thickening

In the general population, both tunica intima and media become thicker with age, which is correlated to the age-dependent increase in blood pressure. Under physiological conditions, elevated blood pressure increases the arterial lumen and causes an adaptive increase of intima media thickening (IMT), both due to the hypertrophy of VSMCs and increased extracellular matrix[173]. The IMT can easily be assessed using high resolution ultrasound, measuring the distance between the intima-lumen interface and the media-adventitia interface. The most common locations for IMT-measurements are the carotid arteries (cIMT), often the common carotid artery. Although cIMT is an unspecific marker of arterial damage, it is recognized as a surrogate marker for atherosclerosis[174].

2.6.1.1 cIMT and reference data

The largest study presenting normative data on cIMT in healthy children was published in 2013 by Doyon et al., investigators of the Cardiovascular Comorbidity in Children with Chronic Kidney Disease (4C) Study[175]. They reported reference values for cIMT in 1155 children aged 6-18 years and provided calculation of z-scores according to age or height. Reference values were previously only available for children aged 10 years and older[176].

2.6.1.2 cIMT in CKD

CKD: Even in the milder stage of CKD, cIMT is increased[31 177] and young adults who developed CKD in childhood also have increased cIMT in comparison to controls[178].

CKD-T: While renal transplantation attenuates IMT, it is still higher in pediatric CKD-T patients than in healthy children[38 177 179 180].

2.6.1.3 cIMT and CVD

A recent meta-analysis concluded that cIMT measurements alone could not predict CV events in the general population, but adding carotid plaque detection allowed the prediction of CV events such as myocardial infarction and stroke[181]. It is known that atherosclerosis starts in childhood preceding the occurrence of CV outcomes in the adult patient[22 182 183]. Thus, early recognition of vascular changes to prevent CVD is warranted. So, in patients with elevated CV risk, it is suggested that cIMT measurement should be included in assessment of CV status[22 184 185].

2.6.2 Vascular Calcification

In contrast to calcifications of atherosclerotic plaques in the vascular intima that develops with age in patients with normal renal function, vascular calcification in the uremic milieu develops primarily in the vascular media[186 187]. Medial calcifications decrease the elasticity and compliance of the arteries. The resultant increase in arterial stiffness and loss of arterial resilience exposes the myocardium, brain, and kidneys to higher pressure fluctuations which is a strong predictor of CV morbidity and mortality in patients on chronic dialysis[188].

Vascular calcification is an organized process that simulates the mineralization of bone tissue. Under conditions of increased calcium-phosphorus levels, VSMCs are phenotypically transformed and start expressing mineralization-regulating proteins[189]. In patients undergoing dialysis treatment, VSMCs have been shown to undergo apoptotic cell death[189]. Coronary artery calcification (CAC) visible with CT scan is an early marker of increased CV mortality in adults with dialysis treatment[186]. While there are very few studies of vascular calcification in pediatric CKD, some studies have revealed CACs in dialysis or renal transplant recipients but not in CKD before dialysis[190-192]. CACs were more common in young adults with childhood onset RRT than in children[178 193 194].

3 AIMS OF THE THESIS

The overall objective of this thesis is to investigate the CV health and risk factors for CV morbidity in pediatric CKD and CKD-T patients.

The specific aims of the included Papers are:

- To study the prevalence of known traditional and uremia related CV risk factors in pediatric CKD (Paper I).

- To study the associations of these risk factors in relation to cardiac remodeling, cardiac function and carotid intima media thickening in pediatric CKD and CKD-T (Paper II).

- To study longitudinal changes in cardiac remodeling and function and assess clinical predictive risk factors in pedatric CKD and CKD-T (Paper III).

- To study prospective patterns of CKD-MBD estimated by FGF23 excess and Klotho deficiency, and associations to cardiac remodeling and function as well as carotid intima media thickening in pediatric CKD and CKD-T (Paper IV).

4 MATERIALS AND METHODS

4.1 STUDY POPULATIONS

Papers I-IV:

This thesis presents data from two cohorts (Italy and Sweden) including a total of 58 children with CKD, 44 children with CKD-T and 53 reference children. The CKD children were all equally required to have a GFR ≤75 ml/min/1.73m² and the reference children had a normal renal function.

Paper I:

Children with CKD treated at the Pediatric Nephrology Department in Gaslini Children´s Hospital, in Genoa, Italy were recruited in the years 2002-2003. The inclusion criteria were: current conservative treatment, age 3-18 years and GFR ranging from 10-75 ml/min/1.73m². The exclusion criteria were: unstable clinical condition, acute infection within 3 weeks prior to enrolment and patients with lupus erythromatosus, amyloidosis, primary hyperoxaluria, hepatic insufficiency, malabsorption syndrome, diabetes mellitus, treatment with growth hormone, corticosteroid or other immunosuppressive treatment, or pregnancy. The exclusion criteria were set as they were considered likely to affect the results in terms of inflammation and malnutrition. All patients and their parents approved of the study prior to enrolment.

The reference children were patients with normal renal function seen at the outpatient clinic for check-up of isolated hematuria (no proteinuria). They were all in good health.

In total, 44 CKD patients and 44 reference children accepted the study. 18 CKD children and 10 controls were excluded because they did not meet all the inclusion criteria for reasons listed in Table 2. The final study population consisted of 26 CKD and 34 reference children.

Table 2 Included and excluded study participants Paper I.

	Patients	**Reference**
Approved of the study:	**44**	**44**
Reasons for exclusion:		
- Mild hypertension		2
- Too old	5	
- Present GH therapy	5	
- GFR too high	3	
- GFR too low		1
- Current dialysis treatment	3	
- Lack of information	1	7
- Diabetes	1	
Total included:	**26**	**34**

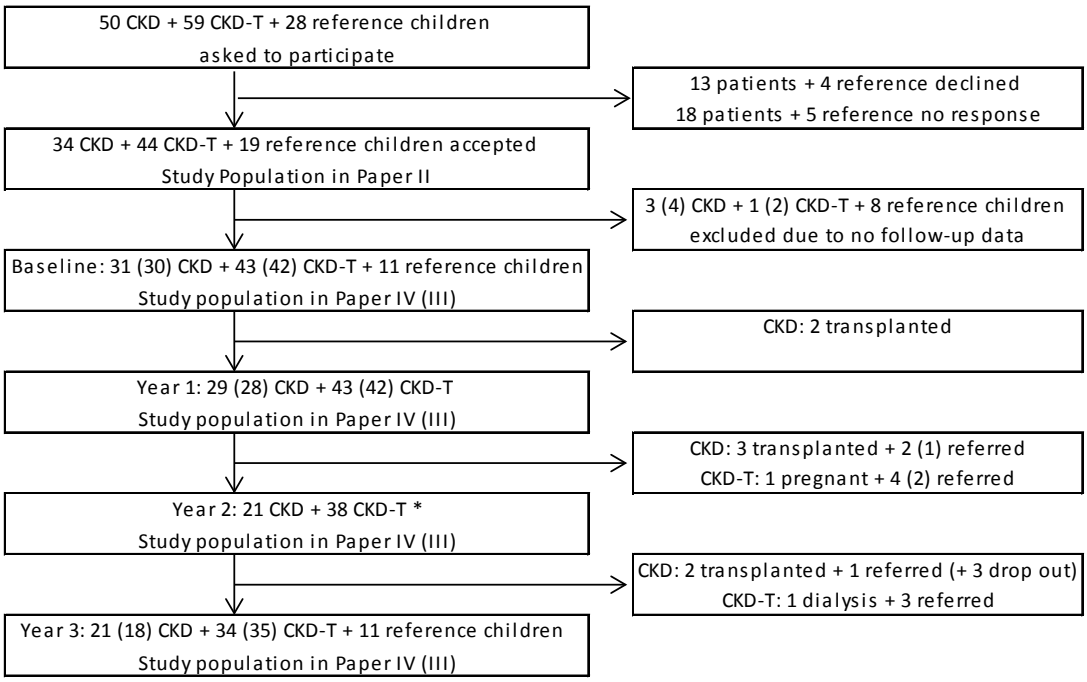
Papers II+IV:

Children with CKD and CKD-T treated at the outpatient clinic at Astrid Lindgren Children´s Hospital, Karolinska University Hospital in Huddinge, Sweden, were recruited between the years 2007 and 2008. Eligibility criteria for the study were 1) age ≤18 years and 2) a functioning renal transplant or CKD with GFR ≤75 ml/min/1.73m². Exclusion criteria were: ongoing dialysis, congenital or primary myocardial disease, overt heart failure, HIV, Hepatitis C or unstable clinical condition as well as present infections within the last 3 weeks.

The reference children were patients in good health and with normal renal function seen at the outpatient clinic for reasons other than reduced renal function. These children were recruited in the years 2007-2008 and 2011. They were matched for age and sex and only one reference child was on any medication (prophylactic antibiotics for recurrent urinary tract infections). They were excluded if diagnosed with hypertension.

Paper III:

The same cohort as in Papers II and IV, but for those patients with missing echocardiographs at baseline, the inclusion year was set one year earlier (n=11) or one year later (n=6). Only patients with available data at baseline following these adjustments in the study protocol were included in the study. In the prospective studies (Papers III-IV) an additional exclusion criterion was applied to those patients who had less than two years of clinical follow-up. The patient-flows from baseline and over follow-up are demonstrated in Figure 10.



* 3 CKD had missing follow-up at year 2 in Papers III and IV. One CKD-T was missing at year 2 in Paper III.

Figure 10. Study population in Papers II-IV as well as patient flows throughout follow-up. Patient flows for Paper III are in parenthesis when not equal to Paper IV.

4.2 CLINICAL ASSESSMENTS

All clinical assessments were performed annually in all patients (Papers III-IV) apart from measurements of cIMT that were done at baseline and the final year of follow-up (Paper IV). The reference cohort was seen at baseline and the final year (Paper IV).

4.2.1 Anthropometry

In all Papers weight, height and BMI for age percentiles; i.e. z-scores or standard deviation scores (SDS) were assessed. The denomination SDS was used in Papers I and II, while z-scores were used in Papers III and IV. In Paper I, CDC (Center for Disease Control and Prevention) growth charts, based on cross-sectional samples of U.S. children, were used as reference material[195]. In Paper II, height and weight SDS was assessed using Swedish reference material[196], and BMI SDS using French reference material[197]. In Papers III-IV, height, weight and BMI z-scores were assessed using British Growth charts[198], since they were available in the statistical software program used in these Papers (Stata 12.0). This reference was chosen over CDC growth charts (used in Paper I) because the British growth charts allow assessment from 0-23 years and CDC growth charts only cover 2-20 years. In Paper III, Obesity was defined as BMI z-scores ≥1.65 (≥95th percentile) and overweight as z-scores ≥1.04 (≥85th percentile), while Paper I defined obesity as BMI SDS >97th percentile.

Pubertal stage was assessed in Papers I-II using Tanner scores. Tanner staging runs from stage 1 (prepubertal) to 5 (adult) and involves genital development in boys, breast development in girls and pubic hair development in both genders. Prepuberty was defined as Tanner stage 1, puberty as Tanner stage 2-4 and adult as Tanner stage 5[199]. When Tanner scores were not available, pubertal status (prepuberty, puberty or adult) was estimated by analyzing pubertal hormones (LH, FSH, Sensitive Estradiol, Testosterone and sex-hormone binding globulin [SHBG]), assessing growth charts and identifying the presence of closed epiphyseal growth plates on available x-rays, and also identifying time for menarche. Prepuberty was defined as LH ≤0.7 U/L, Testosterone ≤0.5 nmol/L or sensitive Estradiol <25 pmol/L and no presence of growth spurt[200-202]. Puberty was defined when increasing levels of LH, FSH, Testosteron or sensitive Estradiol and decreasing SHBG was detected as well as presence of growth spurt and/or menarche. Adulthood was defined when a flattened growth spurt or closed epiphyseal growth plates was found. These measures are indeed surrogate measures of pubertal stage and Tanner scoring to define puberty in all study participants would have been the ideal scenario.

4.2.2 GFR

There are several ways to assess GFR. In Paper I, GFR was estimated (eGFR), using the Schwarz formula which is based on plasma creatinine and the height of the child: height (cm) x k/plasma creatinine (mg/dL). The constant k was set at 0.55 for children <13 years of age and girls aged 13-18 years and 0.7 for boys aged 13-18 years[203]. In recent years this method to assess eGFR in children has been questioned as it overestimates the true renal function as compared to iohexol clearance in CKD. New guidelines now suggest the use of bedside

eGFR equation instead: 0.413 x [height(m) / plasma creatinine(mg/dL)][204]. This equation was used to assess eGFR in Papers II and IV.

The measured GFR (GFR) was assessed using iohexol clearance. Iohexol (Omnipaque™) is a nonradioactive radiographic contrast agent that is injected intravenously, and filtered in the glomeruli, and not reabsorbed or secreted by the tubules. The plasma clearance of iohexol determines GFR in ml/min/1.73m². Iohexol clearance is widely used in routine diagnostics in Scandinavia, and the method corresponds well with inulin clearance[205] which is recognized as the gold standard for evaluating renal clearance. Inulin clearance is, however, a complicated and time-consuming method and not always suitable in clinical practice. The iohexol concentration in plasma is measured at one or more time-points after injection of the contrast agent (in the contralateral arm) and analyzed by a high performance liquid chromatography method. Iohexol clearance was most commonly used to assess GFR in Papers II-IV, while a few, predominantly CKD-T patients, had GFR assessed using inulin clearance.

In a minority of the patients in Papers II-IV, GFR was assessed using Cystatin C (3.8%-5.5% of all patient measurements at baseline) when no clearance-GFR had been performed. Cystatin C is a protein synthesized by all nucleated cells and is freely filtered through the glomeruli. It is not affected by muscle mass (which affects creatinine) or gender. Cystatin C-based GFR was calculated at the Department of Clinical Chemistry at Karolinska University Hospital in Huddinge using the following formula: at ages <14 years: 92.3 x (Cystatin-C mg/l)$^{-1.2307}$, at ages 14-19 years: 94 x (Cystatin-C mg/l)$^{-1.3517}$, and at ages >19 years: 79.1 x (Cystatin-C mg/l)$^{-1.2321}$.

4.2.3 Blood Pressure evaluation

In Paper I, data on blood pressure (BP) were not collected. In Papers II-IV, an automatic systolic and diastolic blood pressure (SBP and DPB) device (model Accutorr Plus, Datascope Corp, U.S.) was used to obtain office BPs according to standard protocols. Office hypertension was defined as a SBP and/or DBP at or over the 95th percentile indexed to the age, gender and height-specific reference values (the Fourth Report) for each subject, and data were presented as BP z-score[206]. In Papers II-III, ambulatory blood pressure measurements (ABPM) were included. A Model 90207 Space Labs (Redmond, WA, U.S.) monitor was used. The BP was measured every 20 to 30 minutes during day and nighttime. Day (08-20), night (00-06) and mean (00-24) ambulatory systolic and diastolic blood pressure (ABP) were determined and compared with published German reference data from healthy children using LMS reference tables to assess ABP z-scores[207]. Only ABPM profiles with a minimum of 30 recordings in the course of 24 hours were accepted. The reference material allows calculation for systolic or diastolic APB z-scores in ages 5-20 years or between 120 and 185 cm in height. As CKD patients are known to be shorter than their peers, the height specific references were used. One previous study in children aged 3-6 years and with height 100-130 cm had similar APBM as children aged 6-8 years[208]. Consequently, we included patients within this age and height span to assess ABP z-scores. In detail, this was

an issue for in total five CKD and six CKD-T patients who were 100-120 cm tall (Paper III). Their ages were 4-7.8 years.

4.2.3.1 Missing data on ABPM

There were missing data on ABPM in Papers II-III. At baseline in Paper III (8/72) 11.1% of day and (10/72) 13.9% of night ABP were missing. Over the entire 3 year period, 14.7% of mean ABP were missing. Therefore multiple imputations were performed in the multivariate analysis in order not to lose power in the analysis.

4.3 BLOOD SAMPLES

Venous blood samples (5-10 mL) were drawn during a routine clinical visit in the morning following an overnight fast in a standardized manner in Papers I-IV.

In Paper I, the following routine biochemical analyses were made at the local clinical laboratory in Italy: blood Hemoglobin (g/dL), as well as plasma levels of Creatinine (mg/dL), Albumin (g/dL), HCO_3 (mEq/L), IGF-1 (ng/mL), IGFBP3 (ng/mL), GH (ng/mL) and Insulin (µIU/mL). Blood samples were frozen at -20° Celsius (C) and sent to Sweden on dry ice on the 10th of June 2004 for future analysis. Samples were stored in Sweden at -80° C until analysis in the year 2006. Analyses performed in Sweden were: Plasma levels of Interleukin-6 (IL-6: pg/mL) and high-sensitivity CRP (hs-CRP: mg/L) using Immulite 1000 (Siemens Healthcare, Sweden), Fetuin-A (g/L) using enzyme-linked immunoassay (ELISA), and Glucose (mg/dL), Cholesterol (mg/dL) and Triglycerides (mg/dL) using standard biochemical analyses (Konelab clinical chemical analyzer, Thermo Fisher Scientific, USA).

In Papers II-IV, routine biochemical analyses performed at the Department of Clinical Chemistry at Karolinska University Hospital in Huddinge were: Hemoglobin (g/L), Creatinine (µmol/L), Cystatin C (mg/L), Glucose (mmol/L), Calcium (mmol/L), Phosphorus (mmol/L), Albumin (g/L), intact-Parathyroid Hormone (i-PTH: ng/L), Triglycerides (mmol/L), Cholesterol (mmol/L), Low-Density Lipoprotein (LDL: mmol/L), High-Density Lipoprotein (HDL: mmol/L), White Cell Blood Count ($x10^9$/L) and hs-CRP (mg/L). Serum and plasma were also frozen and stored at -80°C. Using Immulite 1000 Insulin-like Growth Factor-I (IGF-1: ng/mL), Insulin (µIU/mL) and IL-6 (pg/mL) were analyzed. Commercial ELISA were used according to the manufacturer´s instructions to analyze; Pentraxin-3 (ng/mL), LL-37 peptide (ng/mL), Vascular Cell Adhesion Molecule-1 (VCAM-1: ng/mL) and Intracellular Adhesion Molecule -1 (ICAM-1: ng/mL) as well as FGF23 (RU/mL) and soluble Klotho (pg/mL). Immunochemistry was used to analyze Luteinizing Hormone (LH: U/L), Follicle Stimulating Hormone (FSH: U/L), Sex-Hormone Binding Globulin (SHBG: nmol/L), Testosterone (nmol/L) in boys and sensitive Estradiol (pmol/L) in girls.

The ELISA method is a technique used to identify and quantify substances, such as peptides, proteins, antibodies and hormones, within a sample. This technique uses antibodies that attach themselves to the substance where present. These antibodies generate a specific color, and the amount of color indicates the quantity of substance present.

4.3.1.1 Definitions and cut-off values

HOMA-IR was calculated using the following equation: [fasting plasma glucose (mg/dl) x fasting insulin (µIU/ml) / 405][51]. Insulin resistance was defined as HOMA-IR levels >3.16 for subjects <16 years and >2.5 for subjects >16 years[209], while hyperinsulinemia was defined as fasting insulin ≥20 µIU/mL. Hypercalcemia was defined as albumin-adjusted calcium >2.6 mmol/L in children aged 1-17 years and >2.5 mol/L if 18 years or older. Hyperphosphatemia was defined as phosphate >1.8 mmol/L at ages 1-3 years, >2.0 mmol/L at ages 4-10 years, >1.6 mmol/L at ages 11-17 years and >1.5 mmol/L for females 18 years or older and >1.6 mmol/L for males. Secondary hyperparathyroidism was defined as i-PTH >65 ng/L. Cut-off values to assess FGF23 excess or Klotho deficiency were set at 101 RU/mL[115] and ≤765 pg/mL[116], respectively.

4.4 CARDIAC ANALYSES

In Papers II-IV, echocardiography was performed using commercially available equipment (Vivid 7, GE, Vingmed, Horten, Norway). All children were examined with two-dimensional (2D) and M-mode echocardiography following the recommendations of the European and American Society of Echocardiography[210]. 2D-imaging allows structures to be viewed in a cross-section of the heart and images were acquired from the apical four (A4C) and two chamber (A2C) as well as parasternal views. M-mode analysis provides high temporal and spatial resolutions with the ability to focus on one of the lines from the 2D-trace. Baseline measurements were made within six months from the inclusion date and in subsequent years at the same clinical appointment as the other investigations.

Each child was examined lying down in a lateral cubitus position and all images were recorded at the time of end expiration. The collected data were stored and analyzed offline with commercially available software (EchoPac, GE Medical, Horten, Norway) by two investigators (GV: analyzing cardiac geometry and YTL: analyzing PWD and TDI measures of cardiac function).

4.4.1 Cardiac geometry

End-diastolic left ventricular internal dimension (LVIDd), posterial wall thickness (PWTd) and septal wall thickness (SWTd) were measured from 2D-imaging with M-mode analysis, Figure 11. End-diastole can be defined as the onset of the QRS complex, but is preferably defined as the time frame following mitral valve closure after atrial contraction in which the cardiac dimension is the largest. Left ventricular internal dimension at end-systole (LVIDs) was also assessed. End-systole is best defined as the time frame preceding mitral valve opening in which the cardiac dimension is smallest in a normal heart.

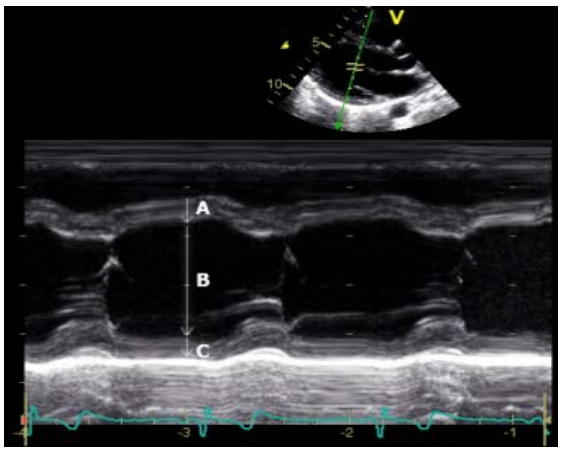


Figure 11. M-mode echocardiography image of the left ventricle in parasternal axis view.
A=Septal Wall Thickness in diastole (SWTd). B=LV Internal Diameter in diastole (LVIDd). C=Posterior Wall Thickness in diastole (PWTd).

The LV mass (LVM) calculations were made using the following formula: 0.8 x [1.04 x (LVIDd + PWTd + SWTd)^3 – (LVIDd)^3] + 0.6g. [210 211] Left ventricular mass index (LVMI) was assessed as: LVMI = LVM / (height in meters)$^{2.7}$.[212] The relative wall thickness (RWT) was calculated using: (PWTd + SWTd) / LVIDd. In Paper III, LVMI z-scores was also established using the reference material published by Foster et al.[135]

Regarding the prevalence for LVH, the cut-off set at 38g/m$^{2.7}$ as presented by Matteucci et al. was used in Paper II[80]. Paper III used the cut-off set at the 95th percentile with reference data published by Khoury et al.[136] and also compared results to other commonly used definitions of LVH[80 135].

4.4.2 Systolic and diastolic function

In order to assess LV systolic function, left ventricle volumes were measured and the ejection fraction (EF) was calculated using the guidelines of European and American Society of Echocardiography[210]. The EF represents the percentage of end-diastolic volume ejected during systole, and is normal if ≥55%[210]. Volume calculations using the Simpson formula in 2D-images of A4C and A2C views at end-diastole and end-systole were used to assess end-diastolic volume (EDV) and end-systolic volume (ESV), where EF was calculated as: EF = (EDV - ESV) / EDV. In addition to EF, peak systolic velocity s´ was assessed using cc-TDI. Previous studies report that while s´ and EF are highly correlated, s´ detect changes earlier than EF[213].

Transmitral blood flow velocity profiles obtained from the PWD A4C view was analyzed by placing a 5 mm sampling volume just below the tips of the mitral leaflets. During diastole, early peak velocity E (passive filling of the ventricle) and late peak velocity A (active filling due to atrial contraction) in m/sec were assessed, Figure 12. The mean velocity peaks from three consecutive heart beats were documented and the ratio E/A was also recorded.

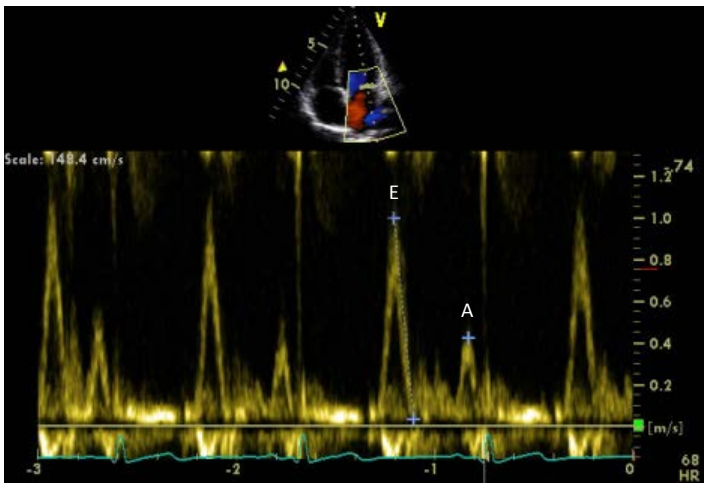


Figure 12. Echocardiographic image of a 4-chamber view showing PWD velocities over the mitral valve. Peaks denote E (early diastole) and A (late diastole) in m/sec.

Color-coded TDI (cc-TDI) loops were also obtained in the A4C and A2C views. Peak systolic (s′), early diastolic (e′), and late diastolic (a′) velocities were measured in cm/sec within a 6 mm circular sample volume following a temporal filtration of 30 milliseconds, Figure 13. Myocardial velocities from three consecutive heartbeats were averaged and displacement in the basal septal, lateral, inferior and anterior mitral annular positions were used in Paper II, while in Papers III and IV only septal and lateral mitral annular sites were used. The ratios of e′/a′ and E/e´ were assessed[147].

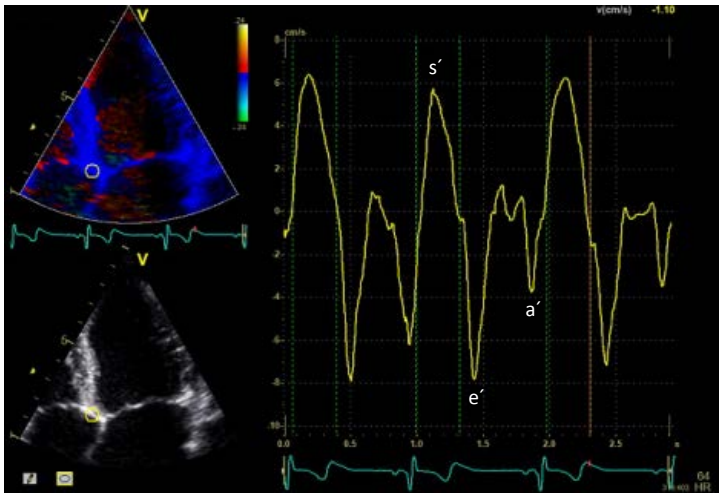


Figure 13. Color-coded TDI echocardiography in 4-chamber view showing myocardial velocities. Peaks denote s´ (systole), e´ (early diastole), and a´ (late diastole) in cm/sec.

The only existing publication on reference values for cc-TDI in children was used to assess z-scores for PWD E, A, E/A as well as TDI e´ and a´[158]. It was not possible to calculate z-scores for cc-TDI e´/a´ or E/e´ ratios using data from this publication.

4.4.2.1 Definition of LVDD

LVDD was defined as cc-TDI e´ or c-PWD E below -2 z-scores.

4.4.2.2 Missing echocardiographic examinations

In Paper II, echocardiographic examinations were available in 15 of 19 reference children, 28 of 34 CKD patients and 30 of 44 CKD-T children. Overall 24.7% of the echocardiographic data were missing.

In Paper III, there were no missing echocardiographic examinations at baseline. Overall, missing values on the echocardiographic outcome variables during follow-up totaled 8.7%.

In Paper IV, missing values on echocardiographic outcome variables during follow-up totaled 11.9%.

4.5 CAROTID INTIMA MEDIA THICKENING

The right and left carotid arteries were examined with a duplex scanner (Sequoia, Siemens Acuson, Mountain View, Ca, USA) using a 6 MHz linear array transducer within six months of the inclusion date by a single trained sonographer. The follow-up examination was performed at the final year in the study. The patients were examined in a supine position with the head slightly turned from the sonographer. The far wall of the common carotid artery, 0.5 to 1.0 cm proximal to the carotid bulb, was used for measurements of the thickness of tunica intima and media; carotid intima-media thickness (cIMT). The lumen diameter (LD) was also measured. The examinations were digitally stored for subsequent analyses by a computer system[214] with automated tracing of echo interfaces and measurements of distances between the wall echoes within a 10 mm long section of the common carotid artery in late diastole, defined by a simultaneous electrocardiographic recording. The mean values of the cIMT and LD were calculated. The intra-observer variability of the same sonographer on similar material has already been published elsewhere[179].

Although it is recommended[185], it was not possible to assess z-scores for cIMT in Study II as present normative data only include children from above the age of 10 years[176 177], and this cohort also involved younger children.

4.5.1.1 Missing data on cIMT

In Paper II, available data on cIMT were as follows: (16/19) 84.2% in the reference group, (28/34) 82.3% in CKD, and (28/44) 63.6% in CKD-T patients. In Paper IV there were overall 73% available data for cIMT in patients at baseline and 85.5% at follow-up.

4.6 STATISTICAL METHODS

The statistical methods used in this thesis, and the variables included in all models, are described in detail in each of the four Papers. Statistical analyses were performed using SAS (SAS Institute, Cary, N.C., USA, version 9.1) in Paper I and Stata software (Statacorp, Texas, USA, version 10.0 and 12.0) in Papers II-IV. All variables were initially assessed for Gaussian distribution by plotting histograms and Shapiro-Wilk testing. Results are expressed as means (SD) for approximately normally distributed variables, and median (range) for non-normally distributed parameters.

Cross-sectional analyses of between-group differences were analyzed using t-test or Wilcoxon-test for two group comparisons, and ANOVA or Kruskall-Wallis test followed by Scheffé- or Mann-Whitney U-post hoc testing for three group comparisons, where appropriate. Spearman correlation coefficients were calculated for univariate analysis. Non-normally distributed variables were log-transformed (using base e) for parametric testing. In Paper II, a multiple stepwise forward linear regression analysis was performed to assess independent associations to the outcome variable of choice. Missing data for linear regression analysis were imputed by multiple imputation methods. The Chi-square test was used to investigate differences in proportions of categorical variables. ANOVA repeated measure was used for intra-observer variability analyses, and ICC (intra-class correlation) to assess inter-observer variability and results presented in percentage.

In Papers III and IV, longitudinal dependent data analysis of comparisons between two time points was analyzed with paired t-test or paired Wilcoxon where appropriate. For further longitudinal analyses both generalized estimating equations (GEE) and linear mixed models were used. The linear mixed models included a random subject effect, adjusting for repeated measurement of the same subject at different visits. The mixed model is a linear regression model that takes into account dependency derived from repeated measures of one person (longitudinally) and has advantages if the dataset includes some missing data. Missing data for ABPM (Paper III) were assessed as missing at random and accounted for using multiple imputations. GEE was used to analyze whether longitudinally calculated z-score variables differed from a healthy reference population. Fixed effects models or paired Wilcoxon tests were used to analyze differences in those patients being transplanted during follow-up. Exponential relationships between two variables were modeled in a linear regression using restricted cubic splines with three knots (Paper IV).

A p-value <0.05 was considered statistically significant in all Papers.

4.7 INFORMED CONSENT AND ETHICAL CONSIDERATIONS

The study protocols comply with the declaration of Helsinki. The children and parents gave informed consent before inclusion (Papers I-IV). All studies have been approved by the local ethics committees.

5 RESULTS

5.1 SUBJECT CHARACTERISTICS

A summary of patient characteristics in the four Papers are presented in Table 3.

Table 3 Patient characteristics in Papers I-IV.

	Paper I	**Papers II + IV**		**Paper III**	
CKD or CKD-T, n	CKD, 26	CKD, 34*	CKD-T, 44**	CKD, 30	CKD-T, 42
Male, n	16(61.5)	23(67.6)	24(54.5)	19(63.3)	23(54.8)
Age, years	10.6±4.3	9.9±4.5	12.3±4.4	9.8±4.4	11.8±4.3
Years with CKD	X	4.6[0.80-14.5]	11.0[1.8-17.4]	4.5[0.80-14.5]	10.4[1.8-17.4]
Years with transplant	X	X	5.0[0.92-16.3]	X	5.0[0.90-16.3]
Weight, z-score	-0.9±1.1	-0.07±1.1	0.14±1.1	-0.07±1.0	0.29±1.1
Height, z-score	-1.1±1.1	-0.55±0.90	-0.97±0.91	-0.30±0.96	-0.64±0.79
BMI, z-score	-0.2±1.1	0.35±1.5	1.1±1.1	0.15±1.2	0.83±1.0
GFR, ml/min/1.73m²	42.9±17.8	35.2±19.0	57.1±21.6	35.3±18.3	60.3±18.8
Albuminuria[a], n	X	22 (64.7)	16 (36.4)	18(60.1)	12(28.6)
SBP[b], z-score	X	0.64±1.2	0.74±1.0	0.67±1.2	0.83±0.96
DBP[c], z-score	X	0.59±0.87	0.45±0.83	0.61±0.92	0.45±0.87
Office HT[d], n	X	7(22.6)	9(20.9)	8(26.7)	11(26.8)
Systolic ABP[e], z-score	X	0.08±1.6	0.62±1.3	0.12±1.2	0.53±1.1
Diastolic ABP, z-score	X	0.48±1.6	0.50±1.1	0.02±1.2	0.37±1.0
Ambulatory HT, n(%)	X	3(13.0)	7(16.7)	2(9.5)	5(12.8)
1 antihypertensive, n	13(50.0)	12(35.3)	17(38.6)	11(36.7)	15(35.7)
2 antihypertensives, n	2(7.7)	4(11.8)	3(6.8)	4(13.3)	5(11.9)
3 antihypertensives, n	0	2(5.9)	5(11.4)	2(6.7)	4(9.5)
Phosphate-binders, n	12(46.2)	17(50.0)	6(13.6)	X	X
Vitamin D supplement, n	22(84.6)	15(34.1)	6(13.6)	X	X
Erythropoietin, n	3(11.5)	9(26.5)	4(9.1)	X	X
Immunosuppressives, n	0	4(11.8)	44(100)	2(6.7)	42(100)
GH[f] treatment, n	0	4(11.8)	2(4.6)	5(16.7)	1(2.4)

Values expressed as number (%), mean ±SD or median [range].
* 3 CKD patients excluded in Paper IV (n=31), otherwise the same cohort
** 1 CKD-T patient excluded in Paper IV (n=43), otherwise the same cohort
[a] Albuminuria ≥20mg/L
[b] SBP = Office systolic blood pressure (data from Paper IV)
[c] DBP = Office diastolic blood pressure (data from Paper IV)
[d] HT = Hypertension (data from Paper IV)
[e] ABP = Ambulatory blood pressure
[f] GH = Growth Hormone

The Italian (Paper I) and Swedish CKD (Paper II) cohorts had similar renal function, but the Italian patients were shorter. As for medications, the major difference was that the Italian children had higher prevalence of vitamin D supplementation compared with the Swedish cohort.

The CKD (n=22) and CKD-T (n=16) patients with present albuminuria in Paper II, had severely and moderately increased albuminuria. The urinary albumin to creatinine ratio was 50.4 [9.1-273.1] mg/mmol in CKD, and 16 [4-364.7] mg/mmol in CKD-T patients.

The characteristics of reference children included in Papers I, II and IV are outlined in Table 4. The Italian reference children were younger, but otherwise comparable. Two reference children in Paper II had present albuminuria. Their urinary albumin to creatinine ratios were low; 5.9 and 1.8 mg/mmol, respectively.

Table 4 Characteristics of reference children in Papers I, II and IV

	Paper I	**Paper II**	**Paper IV**
Reference children, n	34	19	11
Male, n	16 (47.1)	11 (58)	6 (54.5)
Age, years	9.0±3.1	12.2±4.5	11.1±4.5
Weight, z-score	0.5±1.5	0.5±1.3	0.3±1.0
Height, z-score	-0.1±1.3	0.4±1.1	-0.8±0.9
BMI, z-score	0.8±1.0	0.7±1.3	0.2±1.0
eGFR, ml/min/1.73m²	114.2±17.9	109.3±17.9	108.7±12.2
Albuminuria[a], n	0	2(10.5)	0

[a] Albuminuria ≥20mg/L
Values expressed as number (%) and mean ±SD.

In Paper I, patients treated with corticosteroids or immunosuppressive agents were excluded, which might be a possible cause of the varying numbers of patients with glomerular disease in the two cohorts, Table 5. Because of this the number of patients with CAKUT is much higher in the Italian cohort (65.4% vs. 38.2%). The causes of CKD were very similar to those published previously in Sweden[14]. Interestingly, the Italian cohort shows a similar pattern of causes for CKD as presented in a large Italian epidemiological study[13], which supports earlier findings of regional differences in underlying causes of CKD in different parts of the world.

Table 5 Causes of CKD in Papers I and II

	Paper I	**Paper II**	
CKD or CKD-T, n	CKD, 26	CKD, 34	CKD-T, 44
CAKUT, n	17 (65.4)	13 (38.2)	17 (38.6)
Glomerulonephritis, n	0	5 (14.7)	3 (6.8)
Hemolytic Uremic Syndrome, n	0	2 (5.9)	0
Juvenile nephronophtisis, n	2 (7.7)	1 (2.9)	4 (9.1)
Congenital Nephrotic Syndrome	0	0	8 (18.2)
Nephrotic syndrome/FSGS**	0	2 (5.9)	3 (6.8)
Polycystic kidney disease, n	5 (19.2)	1 (2.9)	4 (9.1)*
Ischemia/Vasculitis n	1 (3.8)	3 (8.8)	2 (4.5)
Other, n	1 (3.8)	7 (20.6)	3 (6.8)

* Of these four patients, two have autosomal dominant polycystic kidney disease
**FSGS= Focal Glomerulosclerosis
Values expressed as number (%).

5.2 RISK MARKERS AND RISK FACTORS

5.2.1 Hypertension

The overall data for office BP and ABP levels as well as prevalence of hypertension and use of antihypertensive medication are outlined in Table 3.

Paper I

In Paper I, BP values were not recorded and ultimately not included in the study. Still, information about antihypertensive medication shows that 57.7% of patients in this cohort were treated with one or multiple antihypertensive medications. The most common single-use antihypertensive medication was an ACE-inhibitor, and the most common combined treatment was an ACE-inhibitor and a diuretic.

Papers II-IV

In Papers II and IV, information about BP status was collected by office BPs, with a prevalence of uncontrolled systolic or diastolic hypertension at 21.6% in CKD patients and 20.9% in CKD-T patients (Paper IV). In Papers II and III, mean ABPs were also assessed showing that only 13% of CKD patients and 16.7% of CKD-T patients had ambulatory hypertension (Paper II). When analyzing ABPM data more closely in Paper III, night diastolic blood pressures were high and revealed a higher prevalence of hypertension compared to night systolic and overall daytime levels. In detail, night diastolic hypertension was present in 17.4% of CKD and 28.2% of CKD-T patients compared to day diastolic hypertension (8.3% and 12.5% in the two groups respectively). Importantly, there were overall 14.7% missing values for ABPM in this patient cohort which might have confounded these results.

Regarding treatment for hypertension, 18 (52.9%) of CKD and 25 (56.8%) of CKD-T patients were treated with one or multiple antihypertensive medications, dominated by the use of ACE-inhibitors (69.7%) followed by calcium-channel antagonists (30.2%), angiotensin receptor blockers (18.6%), β-blockers (18.6%) and diuretics (9.3%).

In Paper III, longitudinal unadjusted changes in BPs were demonstrated. In CKD and CKD-T patients, office SBP z-score decreased significantly, while office DBP and ABPs remained unchanged. Specifically, SBP decreased at an annual rate of 0.15 z-scores per year (95% CI: -0.26, -0.05; p=0.005). Analyzing the groups separately, CKD patients revealed a significant decrease in SBP z-score (β=-0.22, p=0.03), and during the same time the prevalence of current antihypertensive treatment increased from 56.7% to 61.1%. The CKD-T patients only demonstrated borderline significantly reduced SBP z-scores (β=-0.12, p=0.06) and the prevalence of antihypertensive treatment remained stable (57.1% both at baseline and the final year of follow-up).

5.2.2 Biomarkers of CV risk

A summary of biomarkers of anemia, dyslipidemia, inflammation and glucose metabolism are described in Table 6.

Table 6. Biomarkers of anemia, dyslipidemia, inflammation and glucose intolerance/insulin resistance in Papers I-II.

	Paper I	p-value*	Paper II		p-value**
CKD or CKD-T, n	CKD, 26		CKD, 34	CKD-T, 44	
Anemia					
Hemoglobin, g/L	124.0±14.0	0.09	119.7±13.5	121.7±12.2	<0.001
Dyslipidemia					
Cholesterol, mmol/L	4.3±0.88	0.44	4.6±0.97	4.0±0.79	<0.005
Triglycerides, mmol/L	0.93±0.34	0.06	1.1±0.61	1.2±0.50	<0.001
Inflammation					
Hs-CRP, mg/L	1.0[0.1-3.9]	0.42	0.46[0.16-8.8]	0.35[0.16-19.7]	0.92
IL-6, pg/mL	1.4[0.5-5.0]	0.92	2.1[0.00-6.50]	1.9[0.22-25.0]	<0.005
Glucose metabolism					
Glucose, mmol/L	4.6±0.53	0.85	4.8±0.56	4.7±0.43	0.97
Insulin, µIU/mL	10.6[5.3-22.7]	<0.01	8.7[2.0-18.6]	11.5[2.0-56.6]	<0.001
HOMA-IR	2.3[1.1-4.4]	<0.005	1.9[0.36-4.4]	2.2[0.24-13.1]	<0.005

*CKD vs reference (n=34), and **CKD and CKD-T vs reference (n=19).
Values expressed as mean ±SD or median [range].

5.2.2.1 Anemia

Papers I-IV

Hemoglobin levels were analyzed throughout all Papers with slightly higher levels in Italian CKD, than Swedish CKD patients; 124.0±14.0 (Paper I), 119.7±13.5 (Paper II) and 120.0±13.6 (Paper III), Table 6. There was a statistically significant difference compared with reference children in Paper II ($p<0.001$), but only a borderline difference in Paper I ($p=0.09$). CKD-T patients in Papers II and III revealed similar levels (121.7±12.2 and 123.3±12.8).

Only 3 (11.5%) of the patients in Paper I received erythropoietin, while as many as 9 (26.5%) of the CKD patients in Paper II had similar treatment. Regarding CKD-T patients, the prevalence of erythropoietin use was 4 (9.1%) in Paper II, Table 3.

When using the age-based cut-offs to define anemia as suggested in the KDIGO guidelines[67], 12 out of 34 CKD (35.6%) and 16 out of 44 CKD-T (36.4%) patients were anemic, Paper II.

In Paper III, hemoglobin levels increased at an annual rate of 1.5 g/L (95% CI: 0.26, 2.8), $p<0.05$ during 3 years of follow-up (unadjusted analysis).

5.2.2.2 Dyslipidemia

Papers I-II

In Papers I and II, the levels of cholesterol and triglycerides were assessed and compared with reference groups, Table 6. In Paper II, levels for LDL and HDL were also analyzed with higher levels of LDL in both patient groups compared with reference ($p<0.01$), while HDL was similar between groups. When converting the data in Paper I to SI-units, for the simplicity of comparing the cohorts, cholesterol and triglyceride levels were rather similar in Italian CKD, Swedish CKD and Swedish CKD-T patients. Still, the cholesterol and triglyceride levels were higher in patients compared with the reference children only in the Swedish cohort and not in the Italian cohort ($p<0.001$ and $p<0.005$ vs. $p=0.44$ and $p=0.06$ in the two countries respectively).

In total 28.1% (9 of 32) CKD and 7.1% (3 of 42) CKD-T patients had high levels of cholesterol using the definition published in the KDIGO guidelines (>5.2 mmol/L)[40], Paper II. Regarding the prevalence of elevated LDL-cholesterol (≥3.4 mmol/L), the results were similar; 21.8% and 4.8% in the two groups respectively.

Paper IV

The lipid profile (cholesterol and triglycerides) did not change significantly over follow-up ($p=0.30$ and $p=0.76$, unadjusted analyses).

5.2.2.3 Chronic inflammation

Papers I-II

There are discrepancies with regards to levels of inflammatory markers (hs-CRP and IL-6) in Papers I and II, Table 6. In Paper I, there was no difference between patients and controls regarding these biomarkers. In Paper II, IL-6 was higher in both CKD and CKD-T patients compared to the reference group (overall $p<0.005$). The levels also seem to be higher compared to that of CKD patients in Paper I (the Italian cohort). Surprisingly, while there was no difference in hs-CRP between patient and reference groups in Paper II, these patients reveal lower hs-CRP levels compared to patients in Paper I. A possible explanation could be differences in analyzing techniques used. Another possible explanation is selection bias in the Italian cohort. However, the levels are still overall low in both cohorts.

In Paper II, other inflammatory biomarkers were also analyzed; Pentraxin-3, LL-37 and WBC. There was no difference in Pentraxin-3 between the groups ($p=0.60$), but LL-37 and WBC were significantly higher among CKD-T patients compared to reference group ($p<0.001$ and $p<0.005$). There was no difference between CKD patients and the reference children with regard to these biomarkers.

Paper IV

In unadjusted analysis, log hs-CRP increased by 0.10 per year in all patients (p=0.01), but when analyzing CKD and CKD-T patients separately, the increase was not significant in any group (p=0.15 in CKD and p=0.06 in CKD-T).

5.2.2.4 Abnormal Glucose metabolism

Papers I-II

In Papers I and II, fasting glucose and insulin levels were analyzed, and HOMA-IR was calculated in order to assess insulin resistance, Table 6. In Paper I, diabetic children were excluded from the study, while in Paper II diabetic patients (1 child) were only excluded for analyses including glucose and insulin values. All patients were normoglycemic and there were no differences compared with reference groups (p=0.85 and p=0.96). Still, the insulin levels were higher in all patient groups compared to controls; p<0.01 for the Italian cohort (Paper I) and p<0.001 for the Swedish cohort (Paper II). The prevalence of hyperinsulinemia was almost the same in Italian CKD and Swedish CKD-T patients (15.3% vs. 14.3%) but not present at all in Swedish CKD patients. Further HOMA-IR levels were also higher in all patient groups compared to controls; p<0.005 for both the Italian and the Swedish cohorts. The prevalence of insulin resistance was lowest in Swedish CKD patients (14.3%) and higher in Italian CKD (23.1%) and, as expected, highest among CKD-T patients (32.5%). Insulin levels in Italy were analyzed at the local clinical laboratory, while analyses in Sweden were done at the research lab. Differences in methodologies used could explain these differences between countries.

It is important to assess pubertal stage when analyzing insulin resistance as this tends to increase throughout puberty (Tanner stage 2-4) and normalize in adults. In the Italian cohort 8 (30.8%) had Tanner stage 2-4, while the corresponding number in Swedish CKD patients was 8 (23.5%) and in CKD-T 16 (36.4%). The differences in pubertal status might to some extent explain differences in HOMA-IR between the groups. Another important confounder regarding insulin levels is obesity. However, the weight SDS was lower in Italian CKD compared to Sweden; -0.9±1.1 vs. -0.07±1.1, with similar results for BMI SDS so the impact of differences in body composition on the results is thought to be minimal. Further, as many as 84.6% of CKD patients in the Italian cohort and only 34.1% of the Swedish cohort were treated with vitamin D supplementation, which is thought to increase insulin levels[215]. However the evidence for such a causal relationship is not consistent through literature[216].

Paper IV

During follow-up both insulin and HOMA-IR remained unchanged in all patients (p=0.14 and p=0.18, unadjusted analyses).

5.2.2.5 CKD-MBD

Paper IV

While Calcium, Phosphorus and Klotho levels were similar between CKD and CKD-T patients and reference children, PTH and FGF23 levels were elevated, Table 7.

Table 7. Markers of CKD-MBD in CKD, CKD-T and reference children in Paper IV.

	CKD	CKD-T	Reference	p-value
	n=31	n=43	n=11	
Calcium, mmol/L*	2.41 ± 0.10	2.41 ± 0.10	2.36 ± 0.10	0.16
Phosphate, mmol/L	1.47 ± 0.29	1.36 ± 0.23	1.35 ± 0.22	0.14
Intact-PTH, ng/L	106 [20-391]	64 [19-195]	41 [28-80]	<0.001
FGF23, RU/mL	175 [68-1225]	114 [45-3809]	59 [39-82]	<0.001
Klotho, pg/mL	1417.5 [501.4-3314]	1918 [434.7-4515.5]	1746 [671-3438]	0.23

*Albumin-adjusted Calcium

Values expressed as mean ±SD or median [range].

The prevalence of FGF23 excess (70% and 65%) and elevated PTH (75.9% and 47.5%) was high in both CKD and CKD-T patients respectively, while the rate of hyperphosphatemia (9.7% and 4.9%) and hypercalcemia (6.7% and 2.3%) was low. Further, Klotho deficiency was only present in 6.7% of CKD patients and 11.9% of CKD-T patients. Log FGF23 was strongly inversely associated with GFR and increased exponentially in both CKD and CKD-T patients, and reached the threshold for FGF23 excess at GFR 47 ml/min/1.73m², Figure 14.

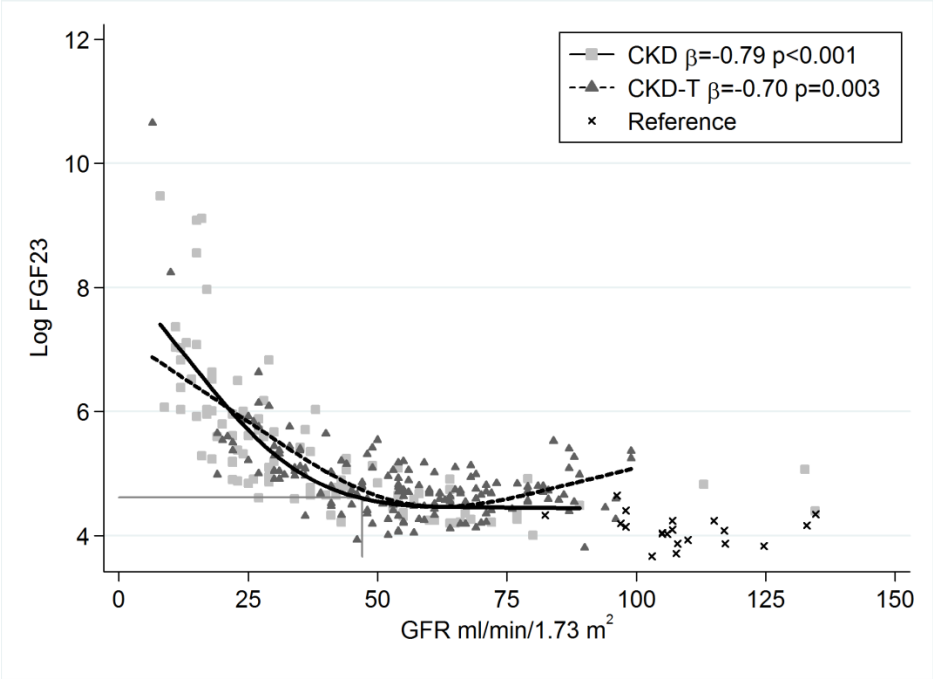


Figure 14. Linear regression analysis for longitudinal correlations between log FGF23 and GFR (ml/min/1.73m²) using Generalized Estimating Equations (GEE) with independence covariance matrix. Data presented graphically using cubic splines with 3 knots due to the non-linear relationship between FGF23 and GFR in CKD, CKD-T and reference children. The vertical line indicates at what GFR level (47 ml/min/1.73m²) mean FGF23 is above the upper reference limit (101 RU/mL = 4.62 log FGF23) for our CKD patients.

There was an association between log Klotho and GFR, only in CKD-T patients, Figure 15.

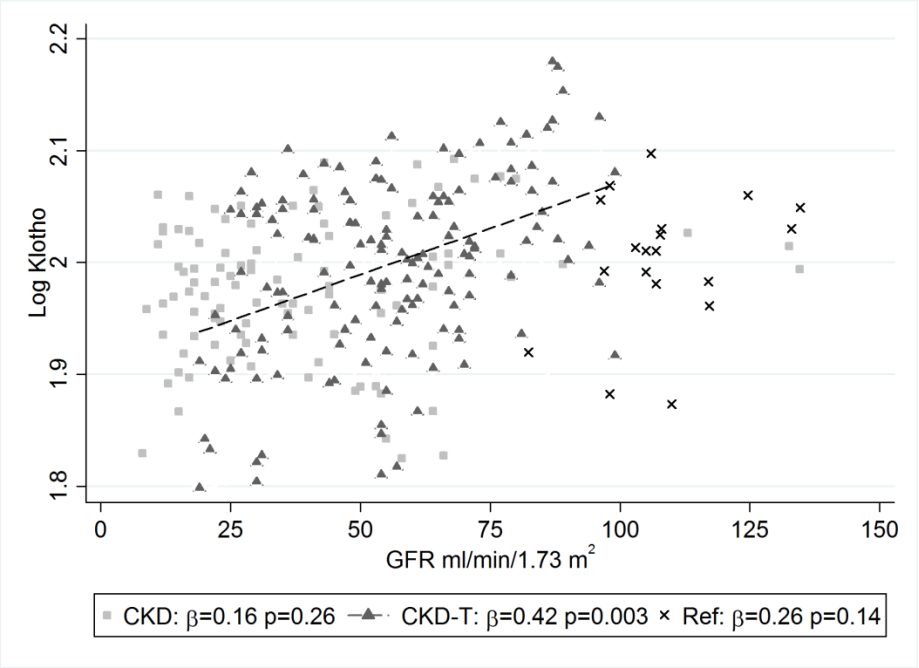


Figure 15. Linear regression analysis for longitudinal correlations between log Klotho and GFR (ml/min/1.73m²) using Generalized Estimating Equations (GEE) with independence covariance matrix in CKD, CKD-T and reference children. The regression line shows significant association in CKD-T patients (β=0.42, p=0.003).

To analyze log FGF23, Klotho, albumin-adjusted calcium, phosphate, and i-PTH as functions of GFR, these variables were plotted versus GFR in CKD and CKD-T patients, Figure 16.

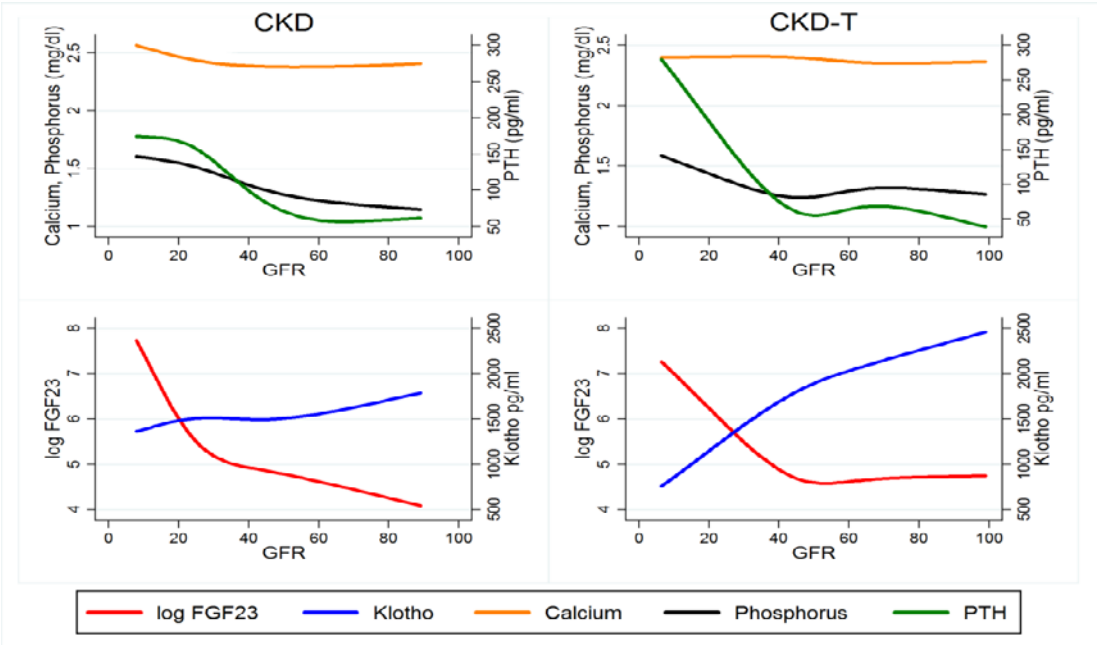


Figure 16. Changes in markers of mineral metabolism with decreasing GFR-values in CKD and CKD-T patients according to a univariate mixed model. The predictions are made for an individual with a random intercept of zero. GFR is modeled using cubic splines with 4 knots.

From these graphs, it appears that FGF23 rise in a similar fashion to i-PTH, but before phosphate or albumin-adjusted calcium increase. Furthermore, when analyzing at which GFR each metabolite had changed significantly when compared to the level set at GFR 89 ml/min/1.73m², this was confirmed. In detail, log FGF23 reached significant change already at GFR 45 and 38 ml/min/1.73m² in CKD and CKD-T patients. Intact PTH reached significant change at GFR 33 and 40 ml/min/1.73m², phosphate at GFR 36 and 21 ml/min/1.73m² and albumin-adjusted calcium at GFR 13 and 26 ml/min/1.73m², in CKD and CKD-T groups respectively. Also, Klotho reached significance at the highest GFR (53 ml/min/1.73m²), but only in CKD-T patients. Klotho did not change significantly at any level of GFR in CKD patients.

In a multivariable model, potential associations for log FGF23 and log Klotho were assessed in CKD and CKD-T patients separately. Following adjustments for age at baseline (years), time of follow-up (years), gender, GFR (ml/min/1.73m²), phosphate (mmol/L), albumin-adjusted calcium (mmol/L), log i-PTH, use of vitamin D supplements and log Klotho, log FGF23 exhibited a mean annual increase of 0.19 ($p<0.001$) in CKD and 0.07 ($p<0.005$) in CKD-T patients over follow-up. Log FGF23 was positively associated to phosphate levels ($\beta=1.25$, $p<0.001$ and $\beta=0.42$, $p<0.01$) in both groups. Albumin-adjusted calcium and GFR were associated with log FGF23 in CKD patients ($\beta=1.52$, $p<0.05$ and $\beta=-0.02$, $p<0.001$), but not in CKD-T patients. Also of interest, the use of vitamin D was associated with log FGF23 in CKD-T patients ($\beta=0.36$, $p<0.005$), but in CKD patients this association disappeared in multivariate analysis ($\beta=0.24$, $p=0.21$). The multivariate model with log Klotho as the outcome parameter was not significant in CKD patients. In CKD-T patients there was a negative correlation between age and Klotho ($\beta=-0.05$, $p<0.005$).

A second model with log FGF23 and log Klotho as outcome variables in absolute values and predictors at baseline was constructed. The only significant predictor for increasing log FGF23 over follow-up was low GFR at baseline in both CKD ($\beta=-0.03$, $p<0.005$) and CKD-T patients ($\beta=-0.01$, $p<0.01$). High GFR ($\beta=0.01$, $p<0.05$) and young age ($\beta=-0.06$, $p<0.01$) at baseline predicted higher log Klotho levels longitudinally in CKD-T patients only.

5.3 CARDIAC REMODELING

5.3.1 LVMI and LVH

Papers II-III

The values for LVMI were higher in Paper III (33.7 and 36.3 $g/m^{2.7}$) as compared to Paper II (28.9 and 31.3 $g/m^{2.7}$) in CKD and CKD-T patients respectively. Consequently, the prevalence of LVH also differs between the Papers, as well as within Paper III when different definitions for LVH were used, in addition to different reference populations, Table 8.

In Paper II, LVMI was significantly elevated in both CKD and CKD-T patients compared to reference children ($p<0.005$). Young age, high BMI z-score and increased systolic ABP z-score were associated with elevated log LVMI in multiple regression analysis in the cross-

sectional cohort. Non-imputed results show (n=52): age (β=-0.02, p<0.005), BMI z-score (β=0.06, p<0.001) and systolic ABP (β=0.05, p<0.001). Following imputation (18 missing data for LVMI and 13 missing values for systolic ABP), the results remained unchanged.

Table 8. Differences in LVMI and LVH prevalence in Papers II and III.

	Paper II		Paper III	
CKD or CKD-T, n	CKD, 29	CKD-T, 30	CKD, 30	CKD-T, 42
LVMI, $g/m^{2.7}$	28.9 [18.9-52.4]	31.1 [21.4-63.4]	33.7 [19.7-53.4]	36.3 [20.4-58.5]
RWT	0.31 [0.24-0.43]	0.36 [0.25-0.46]	0.39 ± 0.02	0.39 ± 0.08
LVM, z-score	X	X	-0.58 [-3.1-1.2]	0.08 [-3.3-2.8]
LVH[a], n	2(6.9)	8(26.7)	5(16.7)	17(40.5)
LVH[b], n	X	X	6(20.0)	10(23.8)
LVH[c], n	X	X	0	3(7.1)

[a] Defined as LVMI >38g/$m^{2.7}$. [80]

[b] Defined as LVMI ≥95th percentile with reference Khoury et al.[136]

[c] Defined as LVM z-score ≥1.65 with reference Foster et al.[135]

Values expressed as number (%), mean ±SD or median [range].

Data in Paper III confirms the results from Paper II, showing that an important predictor for longitudinally increased LVM z-score was a high baseline BMI z-score (β=0.30, p<0.01). Moreover, an increase in BMI z-score (β=0.48, p<0.001) and systolic ABP z-score (β=0.24, p<0.01) during follow-up was associated with an even greater increase in LVM z-score. In these analyses there were no missing data for LVM z-score at baseline, but data for systolic ABP z-score were imputed when missing.

Paper IV

In Paper IV, CKD and CKD-T patients were analyzed separately in the multivariable models. Longitudinally, none of the tested non-traditional CV risk factors, including log FGF23 and/or log Klotho were associated with LVMI or LVH in uni- or multivariate models.

5.4 CARDIAC FUNCTION

5.4.1 LV systolic function

While cc-TDI analyses in Paper II included mean data from four mitral annular sites (septal, lateral, anterior and inferior), the data in Papers III and IV only included mean values from two sites (septal and lateral). This adjustment was made as the only available reference material to assess z-scores exists for septal and lateral sites[158] following current recommendations from ASE[147].

Paper II

There was no significant difference in EF or cc-TDI s´ between CKD, CKD-T patients and reference children. All patients had a normal systolic function as assessed by EF (≥55%).

Paper III

Also in Paper III, EF was also within normal range in all patients. However, cc-TDI s´ peak velocity shows z-scores that are in the low range, Figure 17. In CKD patients, baseline cc-TDI septal s´ z-score was -0.49±1.0, while it was -0.18±1.1 in CKD-T patients. Regarding the lateral side, cc-TDI s´ z-score was -0.61±1.3 in CKD and -0.57±1.2 in CKD-T patients. Four (14.3%) CKD patients and 5 (12.5%) CKD-T patients had lateral s´ below -2 z-scores while the prevalence for the septal side was 2 (7.1%) in CKD patients and 3 (7.5%) in CKD-T patients. This indicates a disturbed systolic function, not yet possible to detect using conventional echocardiographic techniques like EF.

There was no change in unadjusted mean (septal and lateral) cc-TDI s´ peak velocity in CKD or CKD-T patients during follow-up.

Paper IV

In longitudinal multivariable models there was no significant association between EF or cc-TDI s´ and any of our tested CV risk factors.

5.4.2 LV diastolic function

Paper II

The LV diastolic function analyzed using cc-TDI was deteriorated compared to reference children. In detail, cc-TDI a´ peak velocity ($p<0.005$), e´/a´ ($p<0.001$) and E/e´ ($p<0.001$) were worse in both CKD and CKD-T patients as compared to reference children. Regarding cc-TDI e´ peak velocity, the levels in both patient groups were very near significantly different from reference children ($p=0.051$). In contrast, using conventional PWD, there was no difference in E or E/A between the groups. Only PWD A velocity was significantly different in patients compared with the reference group ($p=0.002$).

Increased systolic and diastolic ABP z-score, present albuminuria and young age were all associated with markers of reduced diastolic function using cc-TDI (e´, a´, e´/a´ and E/e´) in multiple regression analysis.

Paper III

Defining left ventricular diastolic dysfunction (LVDD) as normalized lateral cc-TDI e´ below -2 z-scores, the prevalence of diastolic dysfunction at baseline was 7.1% in CKD and 12.5% in CKD-T children. Applying the same cut-off value for septal cc-TDI e´, the prevalence at baseline was higher than for the lateral site; 25.0% in CKD and 20.0% in CKD-T. Also, in similar analyses using PWD E, only 3.3% of CKD and 2.4% of CKD-T patients were diagnosed with LVDD at baseline.

Values for cc-TDI and PWD z-scores of LV diastolic and systolic function during the entire follow-up period were also assessed in comparison to a normal population, Figure 17. This image shows that all variables except PWD E differed as compared to z-score zero.

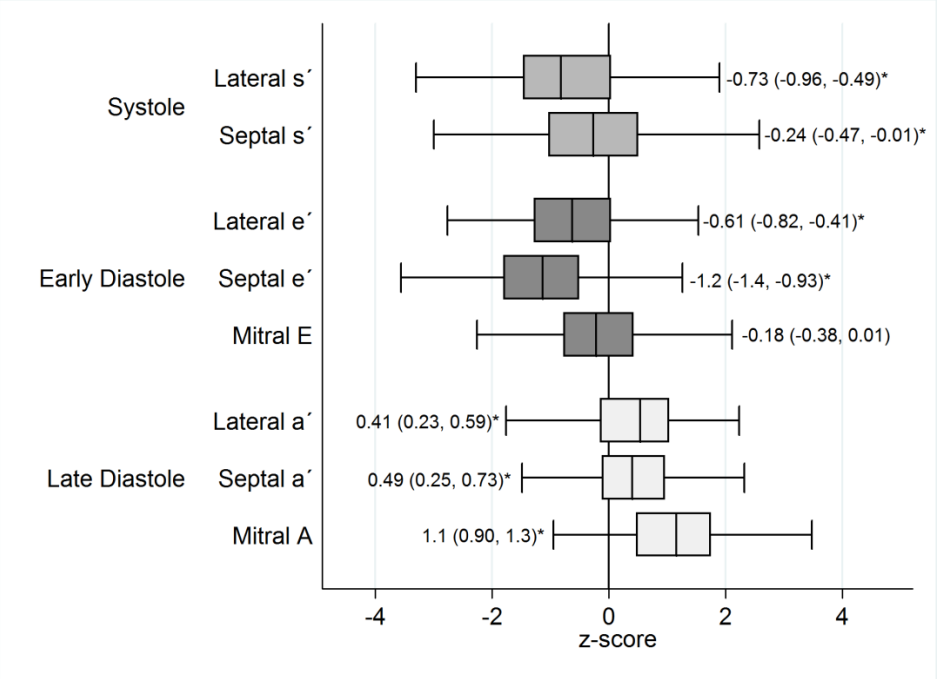


Figure 17. Z-score cc-TDI septal and lateral s´, e´ and a´ as well as z-score PWD E and A were analyzed in CKD and CKD-T patients during the entire follow-up period using GEE (Generalized Estimating Equations). All variables (noted * in the figure), but PWD E was significantly different in patients compared to previously published reference material ($p<0.05$). Numbers documented outside whiskers are group mean with corresponding 95th confidence intervals.

Following adjustments in longitudinal analyses, while cc-TDI e´ peak velocity actually improved over the entire follow-up time, exhibiting a mean annual increase of 0.14 cm/sec ($p<0.05$), cc-TDI a´ worsened with an annual increase of 0.13 cm/sec ($p<0.01$).

CKD-T patients had worse diastolic function compared to CKD patients (cc-TDI e´; $p<0.05$ and E/e´; $p<0.01$). Also, as for LVMI, a lower cc-TDI e´ peak velocity at follow-up was predicted by a young age at baseline. Regarding the importance of renal function, low GFR at baseline predicted a worse cc-TDI e´ during follow-up ($\beta=0.02$, $p<0.05$). In consistency with these data, patients with albuminuria at baseline had lower cc-TDI e´ velocity compared to those without albuminuria ($\beta=-0.50$, $p<0.05$). Moreover, a high systolic ABP z-score at baseline as well as an increase in systolic ABP over time were associated with a worse diastolic function. Specifically, a 1-unit increase in baseline systolic ABP z-score was associated with a decrease in cc-TDI e´/a´ ratio of 0.22, $p=0.01$. Longitudinally, a 1-unit increase in systolic ABP z-score from baseline was associated with 0.18 decrease in cc-TDI e´/a´ ratio, $p<0.01$. The importance of systolic ABP is revealed in Figure 18, showing associations to cc-TDI e´/a´ ratio.

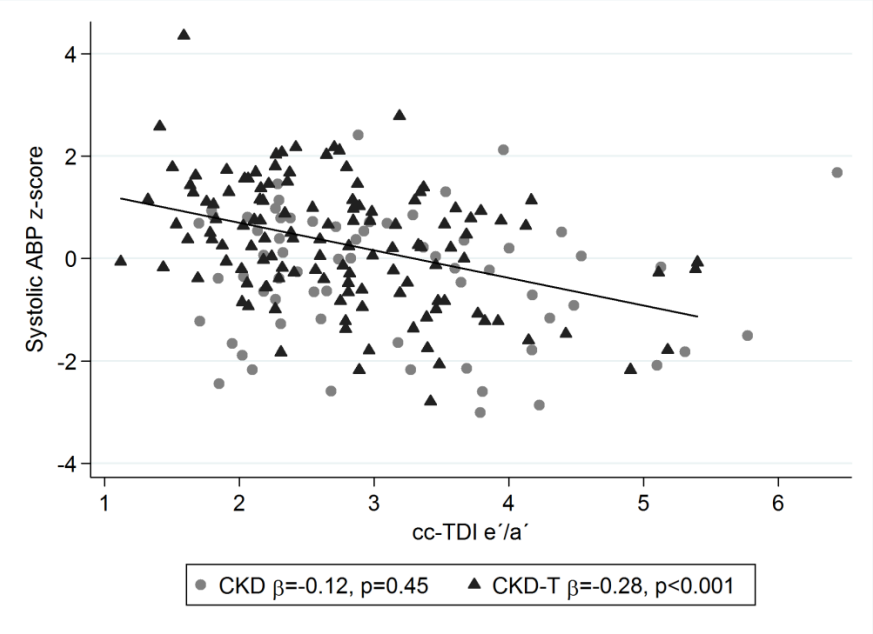


Figure 18. Scatterplot of associations between longitudinal values for cc-TDI e´/a´ and systolic ABP z-score in CKD and CKD-T patients, β=-0.27; p<0.001. In CKD patients only, β=-0.12; p=0.45, and in CKD-T patients β=-0.28; p<0.001. The β and p-values derived from a linear regression with variance estimator of clustered data. The regression line shows significant association in CKD-T patients.

Furthermore, when CKD-T patients were divided into groups by high and low SBP z-score (cut-off at the 50th percentile), LVDD as assessed by lateral cc-TDI e´ peak velocity below -2 z-scores was more prevalent in those patients with high SBP (5/31, 16.1%) compared to those with low SBP (0/9), p=0.03, Figure 19. This underscores the importance of very strict control of BP levels, with optimal targets in the low-normal range.

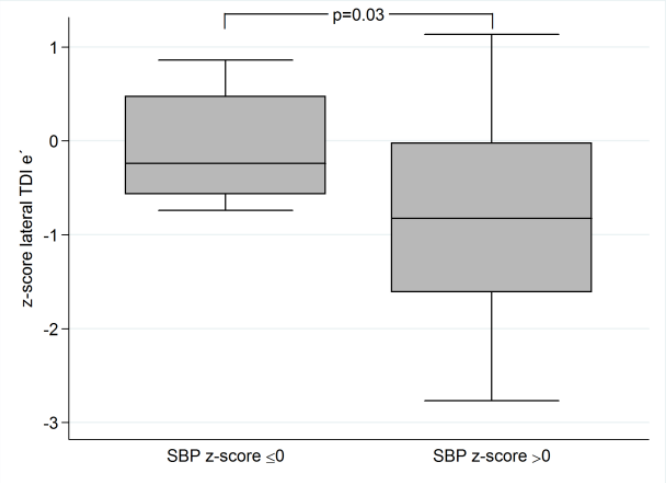


Figure 19. Z-score lateral cc-TDI e´ in CKD-T patients at baseline grouped by Low (n=9) and High (n=31*) Systolic Blood Pressure (≤ or > z-score zero). Data presents results from a paired t-test. *2 missing values for TDI estimates.

Paper IV

In univariate analyses log FGF23 was significantly or near significantly associated to increased LVMI in CKD patients ($p<0.01$) and reduced cc-TDI e´ peak velocity in CKD-T patients ($p=0.06$), this was not true following adjustments. However, in both univariate and multivariable models, log FGF23 (unadjusted $\beta=-0.34$, $p<0.05$ and adjusted $\beta=-0.42$, $p<0.01$ respectively) and log Klotho (unadjusted $\beta=0.35$, $p<0.05$, adjusted $\beta=0.34$, $p<0.05$ respectively) were associated with cc-TDI e´/a´, but only in CKD-T patients. Also, regarding CKD patients, an elevated cholesterol was associated with markers of increased filling pressures (E/e´); unadjusted $\beta=0.60$, $p<0.005$ and adjusted $\beta=0.44$, $p<0.05$.

5.5 CAROTID INTIMA MEDIA THICKENING

Papers II+IV

In Paper II there were no differences in cIMT or LD between reference children, CKD and CKD-T patients. Moreover, in univariate analyses cIMT was not associated with any of the tested CV risk markers in the patient cohort. In a longitudinal multivariable analysis in Paper IV, neither FGF23 nor Klotho or any other markers of CKD-MBD were associated with cIMT.

6 DISCUSSION

6.1 GENERAL DISCUSSION

Bright wrote in 1836, "It is observable, that the hypertrophy of the heart seems, in some degree, to have kept pace with the advance of disease in the kidneys; for in by far the majority of cases, when the heart was increased, the hardness and contraction of the kidney bespoke the probability of long continuance of the disease."[217] Bright was the first to report an association between chronic kidney disease and cardiac abnormalities.

Historically most of our knowledge regarding CKD-associated CVD came from studies in adult dialysis patients, showing that atherosclerotic heart disease was common[1]. However, this end-point is very rare in pediatric patients with RRT, a patient group that further varies in prevalence of CV risk factors and has different causes of CKD compared to adults. In the last decade, more research has focused on the development of CVD in pediatric CKD. From these studies it is clear that the CV system is affected even in children, with the most commonly reported alterations being LVH and increased carotid IMT. Recent reports have also demonstrated signs of LV diastolic dysfunction. Children with dialysis treatment have the worst CV outcome and highest rates of mortality. Even though mortality rates improve dramatically following renal transplantation, they are still elevated compared to the healthy population[3]. Currently, longitudinal studies are warranted to identify important predictors of CKD-associated CVD that could be possible targets for future interventions.

The main findings reported within this thesis are that non-dialyzed pediatric CKD and CKD-T patients reveal sub-clinical signs of CV morbidity with increased LVMI and affected LV diastolic function, while vascular status as revealed by cIMT was normal. Differences in LVMI between the included Papers underscore the variability in estimates derived from echocardiography for these analyses. Furthermore, LV diastolic dysfunction was more easily detected using tissue Doppler imaging compared with pulse wave Doppler, and the most important associated factors were an elevated ambulatory blood pressure and BMI z-scores, present albuminuria, low GFR, and young age. The novel finding that FGF23 and Klotho were associated with a worsened LV diastolic function is of interest and needs to be further explored.

6.2 FINDINGS AND IMPLICATIONS

6.2.1 LVH and LVDD

There are only a few previous prospective longitudinal studies investigating changes in LVMI and predictive risk factors in pediatric CKD and CKD-T[29 30 218-220]. To the best of my knowledge, no study has previously published longitudinal data regarding LV diastolic function using assessments from TDI in this patient group.

The first important consideration is that while LVMI is indeed elevated compared to the reference population in Paper II, the values for LVMI are higher in Paper III compared to

Paper II. Although the cohorts are not examined at the exact same time points, they are otherwise similar. Thus, the variations in LVMI between the cohorts, measured approximately 5 years apart by the same investigator (GV), could possibly be due to intra-individual variations. This may also explain the discrepancy in prevalence for LVH in Papers II and III (6.9% vs. 16.7% in CKD and 26.7% vs. 40.5% in CKD-T, respectively). This is an important finding that has also been recognized by others. Indeed, Schoenmaker et al. revealed that depending on the observer (all pediatric cardiologists), the prevalence of LVH in pediatric patients with RRT ranged from 8-25% using estimates based on LVMI[137].

As previously mentioned the prevalence of LVH also varies extensively between studies on pediatric CKD, which could be attributed to differences in populations examined, index chosen to adjust LVM for body size (BSA, height, weight or lean body mass) as well as reference populations chosen when assessing LVMI percentiles[136] or LVM z-scores[135] and finally the cut-off used to define LVH[80 135 136]. For example, the reference material to assess LVM z-scores by Foster et al. is based on American children using height percentiles[135]. Using this material in Paper III, the patients had low and even negative z-scores and the prevalence of LVH was low (0-7.1% in CKD and CKD-T patients respectively), which was surprising. Similar findings have been shown in a recent Canadian study by McLaughlin et al. revealing negative LVM z-scores and consequently very low prevalence of LVH in children from two years post-renal transplant and onwards[141]. Indeed, Simpson et al. revealed that the reference by Foster et al. show higher LVMI values compared to previous reference material which could be one explanation for this finding[132]. Also, using the reference material by Khoury et al. which includes age percentiles for LVMI, the prevalence for LVH was much higher (20% and 23.8% for CKD and CKD-T respectively), Paper III. Finally, using the cut-off for LVH set at LVMI 38g/$m^{2.7}$, which is the most common definition, but also questioned as LVMI changes with age, the prevalence of LVH was 16.7% and 40.5%, Paper III. However, regardless to which extent, it seems reasonable to conclude that LVMI is indeed increased in pediatric CKD and CKD-T patients compared to reference children, indicating early subclinical CVD.

In addition, Papers II and III demonstrate that both pediatric CKD and CKD-T patients reveal signs of LVDD, whereas the systolic function as assessed by EF was preserved. TDI was more sensitive than PWD echocardiography for identifying LVDD. Indeed, using TDI the prevalence was 7.1% and 12.5% compared to 3.3% and 2.4% using PWD in CKD and CKD-T patients respectively (Paper III). Comparisons of the prevalence of LVDD between studies is difficult as different definitions have been used, however all studies clearly demonstrate a worse diastolic function compared to controls[134 163 166-171]. While a few of these studies have shown an association between LVDD and LVH in pediatric CKD[163 167 168], others have not. LVMI or LVH were not associated with TDI or PWD markers of LVDD in Papers II or III. In the following sections the importance of several CV risk factors in this thesis will be discussed.

6.2.2 Blood Pressure and CVD

In adults, hypertension is a well-known and strong risk factor for CKD. Nevertheless, the cause-effect association can also go in the opposite direction. This is shown in pediatric CKD, where hypertension is rarely the cause of CKD, but rather a result of it. Hypertension can develop even in early phases of CKD, which is likely to increase the risk of CVD. Indeed, hypertension leads to a chronic increase in afterload of the LV and consequently the myocardium thickens in order to work with greater force against this elevated pressure.

Others have confirmed that LVH is more frequent among patients with hypertension and there is support for the fact that LVMI is closely correlated with systolic BP[29 30 81 130 131]. In a two year prospective longitudinal study of pediatric CKD patients with good BP control, prevalence of LVH decreased from 31% to 23%[29]. Furthermore, in a large prospective study from the CKiD cohort, systolic BP and use of antihypertensive medication other than ACE-I and/or ARB, were important predictors of LVH[30]. In support of these findings, plasma angiotensin II, renin and angiotensin-converting enzyme have been shown to correlate positively with LVMI[221]. Also, treatment with ACE-I or ARB was more effective in reducing LVH compared with β-blockers, and also reduced the risk of CV death[33 222]. Similar findings was also published in children and young adults with a renal transplant, where patients with normal BP had much lower prevalence of LVH (14%) compared to those with uncontrolled hypertension (31-44%) and controlled hypertension (37%). These data underlines the risk of elevated BP[223], and also the fact that patients in need of medications to control their BP might be more vulnerable even to slightly elevated BP, in which case targeting lower BPs might be clinically relevant.

Papers II and III presented in this thesis could not confirm a possible protective role of ACE-I and/or ARB use, but do confirm previous and current data showing that an elevated BP significantly predicts cardiac remodeling, and a worse LV diastolic function in pediatric CKD and CKD-T patients. In these studies data from ABPMs were used, which allows for the possibility to evaluate effects of the mean blood pressure over 24 hours, and which rules out possible white coat hypertension while identifying those with masked hypertension. Previous studies have shown that ABPs have better predictive values for LVMI and LVH compared with office BPs[130]. Indeed, there is increasing evidence that mean nocturnal BP level is the most sensitive predictor of CV morbidity and mortality[224]. The results from Paper III indicate that the patient group with highest nocturnal BPs (CKD-T) had the worst LV diastolic function as assessed by TDI (e´ and E/e´). In support of this finding, Mallamacci et al. recently showed that adult renal transplant recipients had high prevalence of nocturnal hypertension and this, but no other BP metrics, was independently associated to elevated cIMT[225].

There seems to be no clear specified cut-off for when an elevated BP becomes dangerous. The patients included in Papers II-IV reveal low prevalence of ambulatory hypertension, but still demonstrate signs of cardiac morbidity. In similarity, Sinha et al. showed that in a cohort of normotensive pediatric CKD patients, LVH was closely associated with BPs even in the

normal to high range[131]. Also, Matteucci et al. showed in a large study with data from the ESCAPE trial that intensified BP control (target BP <50th percentile) did not result in a more prominent decrease in LVMI or lower prevalence of LVH compared to the ordinarily treated group[29]. However, the group with intensified BP control did have a better myocardial systolic function compared to the group with normal BP control, underscoring the effect of BP on the heart even at levels in the normal range[29]. The results from Paper III confirm this finding showing that not only overt hypertension, but also mildly elevated office systolic BP over the 50th percentile is a risk factor for worsened cardiac function, which underscores the importance of very strict blood pressure control.

It thus seems clear that even mildly elevated BP is associated with both increased LVMI and worsened LV diastolic function. Interestingly, recent studies using cardiac magnetic resonance (CMR) imaging have shown that adult CKD stage 2-4 patients, despite normal LVMI and well controlled BP, have signs of myocardial fibrosis which alters cardiac function. So an elevated BP does not seem to be the only important predictor.[162] Indeed, in Paper II, the multivariable models with TDI estimates of LV diastolic function as outcome measure show that only 15%-18% of changes in LV diastolic function could be explained. These findings indicate that other factors than our measured variables might also be of importance for the development of LVDD.

6.2.3 Dyslipidemia and CVD

Dyslipidemia is a profound risk factor for CVD in the general adult and pediatric population. To assess the risk of dyslipidemia in patients with CKD is difficult due to co-existence with other CV risk factors such as inflammation and oxidative stress. Still, dyslipidemia is common in CKD and is thought to contribute to the development of atherosclerosis. This is supported by recent randomized controlled trials (RCTs) showing a positive effect of statin treatment in lowering lipid levels and reducing the risk of major atherosclerotic or cardiac events in in adults with advanced CKD and CKD-T[226 227]. However, the effect has not been confirmed by RCTs in dialysis patients[228], and the effect of statins on mortality is debated[229 230].

The risk of dyslipidemia in pediatric CKD is not clear. In the most recent guidelines regarding lipid lowering treatment in pediatric CKD from KDIGO (2013), it is not recommended to use statins in children or adolescents with CKD due to lack of clear benefit together with safety concerns associated with long-term use in children[10]. They state, which seems reasonable, that it is not advisable to extrapolate results from trials in adults to children for several reasons. The most compelling reason is that the atherosclerotic lesions targeted by lipid-lowering agents are more likely to be present in advanced CKD in adults compared to children. Indeed, few studies have shown correlations between dyslipidemia and atherosclerosis in pediatric CKD. In a study from 1976 by Pennisi et al., an elevated lipid profile was associated with coronary artery disease in pediatric hemodialysis and transplant recipients[231]. In one more recent study, dyslipidemia was associated with an increased cIMT in pediatric CKD[31]. Nonetheless this was not confirmed in Papers II and IV.

Interestingly, in this thesis both cross-sectional (Paper II) and longitudinal (Paper IV) correlations were found between a worse LV diastolic function (E/e´) and cholesterol in CKD patients, being the patient group with highest level of cholesterol. The diastolic function as measured by E/e´ represents increased filling pressures. Thus, speculatively the deranged lipid profile seen in pediatric CKD could be associated with increased LV filling pressures. That cholesterol load, both in serum and myocardium, is associated with worsened cardiac function as assessed by TDI has been shown in experimental studies[232], but the mechanism is yet unknown. Whether pediatric CKD patients with high levels of cholesterol and increased filling pressures would benefit from statin treatment is yet to be explored.

6.2.4 Chronic inflammation and CVD

Chronic inflammation occurs via a cascade of biological pathways that involve the vasculature and immune system, leading to the accumulation of pro-inflammatory mediators in the tissue. The attachment of macrophages to vascular endothelial cells induces endothelial cell injury, potentially resulting in atherosclerosis[233]. The causal role of various inflammatory biomarkers on CVD development in adult CKD remains uncertain, and there are very few studies in children. A recent study presented an association between increased cIMT and inflammation and oxidative stress in pediatric CKD (including non-dialysis and dialysis patients)[234], but this could not be confirmed in Papers II or IV. Furthermore, previous studies in children with CKD have shown conflicting results on the role of inflammation in the development of cardiac morbidity. For example, Matteucci et al. present in a multicenter study of 156 children with CKD stage 2-4, that patients with elevated CRP levels reveal a higher prevalence of concentric remodeling or LVH compared to those with lower CRP levels[80]. Also, Ece et al. showed in a study of pediatric dialysis and non-dialysis CKD children, that inflammation was associated with elevated LVMI, and similar association has been revealed in adult CKD[76 79]. However, these results were not confirmed by others or in Papers II or IV[81 235].

6.2.5 Glucose intolerance, Insulin Resistance and CVD

Insulin resistance is a risk factor for mortality in patients with hemodialysis[236], but is related to CVD even before RRT is commenced[237]. However, renal transplant recipients are those with highest risk for CV events and mortality due to the development of NODAT[53-55]. Very few studies have been published regarding the role of glucose intolerance and insulin resistance on CVD in pediatric CKD. A recent long-term retrospective study of 274 CKD-T patients noted that overall survival was only 75% at 20 years post pediatric renal transplant and NODAT was a significant risk factor for CV events and mortality[238]. However, in a study by Canpolat et al. of 66 pediatric dialysis and non-dialysis CKD patients, neither glucose levels nor HOMA-IR had any impact on vascular or cardiac morbidity[56]. In accordance with these findings, while the patients in Papers I and II demonstrate both hyperinsulinemia and insulin resistance, this did not predict longitudinal cardiac or vascular morbidity (Paper IV).

6.2.6 Anemia and CVD

Regarding the role of anemia in CVD, longitudinal studies in adult CKD patients show that changes in hemoglobin parallel LV growth and are associated with LV systolic dysfunction in dialyzed patients[68 144]. Indeed, anemia is associated with increased risk of mortality and morbidity, principally due to cardiac disease and stroke[239]. However, it should be noted that erythropoiesis-stimulating agents have not been able to show a positive effect on CV-morbidity or mortality, but rather a slightly increased risk[240].

Studies in children with CKD have confirmed the role of anemia in cardiac remodeling and function[30 80 167]. In the study by Kupferman et al., presenting prospective data from the CKiD cohort of 478 children, anemia increased the risk of LVH by more than three times[30]. The plausible mechanism is that anemia leads to an increased preload and thereby thickening of the LV. Despite that anemia was common, the results presented in this thesis could not confirm the importance of low blood hemoglobin on progression in LVMI or on worsened LV diastolic function in pediatric CKD or CKD-T patients (Papers II-III).

6.2.7 Albuminuria, GFR and CVD

In a meta-analysis from the general population, low GFR and present albuminuria were associated with all-cause and CV mortality even following adjustments for other known CV risk factors[241]. The relationship was linear and the CV mortality was twice as high in patients with CKD stage 3 and three times higher at stage 4, compared with individuals with normal kidney function[241]. Regarding albuminuria even levels at the upper end of the normal range conferred elevated CV risk, indicating that even slight increases in albuminuria require clinical attention[241]. In detail, patients aged 30 years with CKD stage 3B or 4, had reductions in life expectancy of around 17 or 25 years, respectively, compared to individuals with normal kidney function[242]. Also, patients in a similar age group with albuminuria stage 2 and 3 were associated with shortening of life expectancy by around 10 and 18 years, respectively, compared to individuals without albuminuria[242]. Endothelial dysfunction has been implicated as a potential mechanism underlying the relationship between albuminuria and CVD[243].

The importance of renal function on cardiac morbidity was shown by Park et al., revealing an inverse relationship between the prevalence of LVH and the level of renal function in a large study of 3487 adults[143]. The prevalence rates of LVH ranged from 32%, 48%, 57%, and 75% for estimated GFR (eGFR) categories ≥60, 45-59, 30-44, and <30ml/min/1.73m^2, respectively. Previous studies in pediatric CKD have also shown a plausible role of low GFR and albuminuria in the development of LVH and worsened LV diastolic function as well as increased cIMT[81 134 177 244]. The structural and functional cardiac response to early and mild reduction in renal function was recently examined using unilaterally nephrectomized (UNX) rats[245]. Cardiac and renal function was assessed at 4 and 16 weeks post UNX. After just 4 weeks, despite no change in BP, GFR and proteinuria, myocardial fibrosis had increased, with signs of LV diastolic dysfunction but preserved EF. An increased rate of apoptosis was also found in myocardial cells. After 16 weeks, the LVMI had increased, LV fibrosis and

diastolic dysfunction had progressed, and EF was also decreased. By this time, BP and proteinuria had increased while GFR decreased. This study shows a correlation between GFR, proteinuria and cardiac remodeling and function, even at very mild CKD.

Papers II and III in this thesis confirm the important role of decreased GFR and present albuminuria as predictors of a worsened LV diastolic function. The exact causal mechanism is yet to be explored; although it is likely that an interaction between several traditional and non-traditional risk factors affect the CV system in multiple ways.

6.2.8 CKD-MBD and CVD

Key modulators of atherosclerosis in CKD have been attributed to decreased calcification inhibitors (like Fetuin-A), hyperphosphatemia, high calcium-phosphate product and parathyroid hormone, but their role has not been elucidated in interventional trials[246]. Also, in the most recent years, two new players in this field; FGF23 and Klotho have generated much interest.

In Paper IV, markers of CKD-MBD were analyzed with emphasis on alterations in FGF23 and Klotho. Longitudinal associations between these biomarkers and renal function were assessed as well as changes following renal transplantation. The results from Paper IV confirm other recent pediatric and adult studies showing that FGF23 excess might be the first sign of disturbed mineral metabolism in CKD[115 120 121].

In studies assessing the impact of mineral disturbances on vascular changes, it has been found that increased calcium-phosphorus level, use of calcium-containing phosphate binders, hyperparathyroidism and calcitriol dose were associated with increased cIMT in pediatric CKD and CKD-T patients[164 177 234 247]. While vascular calcifications are uncommon in non-dialysis children, Shroff et al. revealed that in pediatric dialysis patients, high level of PTH and low Fetuin-A predicted CACs[248 249]. Furthermore, Srivaths et al. revealed in a small study of pediatric hemodialysis patients that progression of CACs was predicted by elevated phosphate and FGF23 levels[250]. Indeed, in this population a disturbed mineral metabolism was one of the most important promoters of vascular calcification, in which disturbances in the FGF23-Klotho axis are thought to play an important role[251].

While experimental studies have confirmed the association between Klotho deficiency and vascular calcification in CKD[110 252 253], the association between FGF23 and vascular calcification is not fully understood. Experimental studies could not see expression of FGF23 in human or mouse VSMCs or in normal or calcified mouse aortas[254]. Instead, higher levels of phosphate were strongly associated with CACs independent of FGF23 levels, and high phosphate induced vascular calcification in vitro[254]. Further, Shroff et al. showed in a study of medium sized muscular arteries in non-dialysis (CKD stage 5) and dialysis patients[251], that longitudinal exposure to elevated levels of calcium and/or phosphate caused vascular calcifications. Calcium and phosphate induced apoptosis of VSMCs.[251] However, in Paper IV, there were no association between markers of CKD-MBD and cIMT, a surrogate marker of atherosclerosis.

Concerning the role of CKD-MBD on cardiac remodeling and function in pediatric patients, small cross-sectional studies have revealed an association to elevated levels of calcium and phosphate as well as PTH[97 134 164]. In addition, Faul et al. showed that elevated FGF23 caused pathological hypertrophy in isolated rat cardiomyocytes via FGF receptor-dependent activation of the calcineurin-NFAT signaling pathway, independent of Klotho[255]. Injection of FGF23 resulted in LVH, and treatment with an FGF-receptor blocker attenuated LVH, although no change in blood pressure was observed. Further, Grabner et al. showed that blocking FGF receptor 4 (FGFR4), inhibits FGF23 induced hypertrophy in isolated cardiomyocytes and attenuates LVH in rats. These results suggest a causal role for FGF23 in the pathogenesis of LVH. In adult non-dialysis and dialysis-dependent CKD, chronically elevated FGF23 levels contribute directly to high rates of LVH and CV related mortality[102 103 106 107]. Also in pediatric dialysis patients, FGF23 has been shown to be associated to LVH[256]. Elevated FGF23 is also associated with cardiac disease in patients with preserved renal function populations[257-259].

In Paper IV, possible correlations between longitudinal levels of FGF23 and Klotho, and cardiac remodeling or cardiac function were analyzed. The results demonstrates, in similarity to Sinha et al.,[138] that FGF23 was not associated to LVMI in pediatric CKD. However, the novel finding that markers of a disturbed diastolic function (TDI e´/a´) correlated with both high FGF23 and low Klotho deserves attention. Mencarelli et al., previously showed that TDI e´/a´ was inversely associated with levels of phosphate, calcium and calcium-phosphate product, underlining a possible role of mineral metabolism in the development of cardiac dysfunction[134]. Also, FGF23 correlated to the degree of congestive heart failure in a small pediatric study[260]. Whether FGF23 and Klotho are true causal factors, or just intermediate risk markers, in the development of cardiac dysfunction needs to be further elucidated in future studies.

Regarding the possible role of vitamin D in CKD-associated CVD, the mechanism is unclear[261]. Nonetheless, vitamin D deficiency is common in CKD and observational studies in adults have shown that vitamin D deficiency is associated with increased risk of CV events. Experimental data also suggests that the vitamin D pathway is involved in modifying cardiac structure and function[262].

6.2.9 Clinical implications

The results from this thesis bring forward findings that are important for the pediatrician in his/her daily clinical work. First, despite a lack of symptoms, children with non-dialysis CKD or CKD-T demonstrate cardiac alterations that might be of importance for future CVD. Today, echocardiography is the preferred choice of cardiac examination because it is safe, cheap, non-invasive, involves no radiation and is available at most clinics worldwide. However, it is important to consider great variations when assessing LVMI and LVH. Thus, caution is encouraged, and the need to standardize indexing for LVM and possibly even a European reference material in order to assess z-scores or percentiles is further acknowledged. The importance of adding more sensitive tools to assess cardiac function, like

TDI to yield more information in order to identify patients at risk for future CVD, is emphasized.

Furthermore, early recognition of potential CV risk factors such as GFR below 60 ml/min/1.73m^2, presence of albuminuria and/or office SBP >50th percentile should targeted. Annual ABPMs are advised in these patients and antihypertensive treatment, preferably using ACE-I and/or ARB, should be commenced in order to decrease the rate of albuminuria, preserve renal function and lower BPs. However, the exact targets for BP and when treatment should be started remain to be established by future research. In the mean-time, current guidelines from the European Society of Hypertension (ESH)[263] and KDIGO 2012[10] recommending to commence antihypertensive treatment at SBP >90th percentile and target ABPs at <75th percentile in general or 50th percentile for those with albuminuria, seems reasonable given the results presented in this thesis.

Finally, although FGF23 and Klotho are not yet analyzed in clinical practice, other markers of CKD-MBD, such as calcium, phosphorus, iPTH and vitamin D, should be measured regularly. Attempts to avoid abnormalities in mineral metabolism should be aimed for, as this might be of importance for future CVD.

6.3 METHODOLOGICAL CONSIDERATIONS

6.3.1.1 Study design

Papers I and II were cross-sectional studies examining differences between children and adolescents with CKD, CKD-T and children with normal renal function with regard to known CV risk markers and factors (Paper I) as well as CV outcome (Paper II). These studies included as many patients as possible. Unfortunately, in Paper I only 26 of 44 patients were available for analysis, with reasons for exclusion listed in Table 1. Altogether 50 CKD and 59 CKD-T patients were asked to participate in Paper II-IV, but only 34 and 44 accepted the study. Furthermore, a rather low number of reference children were accepted in Paper II. The reason was foremost difficulties in recruiting children which is seen as 9 out of 28 (equivalent to 32%), either did not respond or declined participation.

As cross-sectional studies are hypothesis generating, showing correlations rather than predictions, longitudinal studies are important to be able to assess possible risk factors for an outcome. Papers III and IV were designed as prospective cohort studies. Importantly though, in Paper III the baseline year was actually included one year retrospectively or one year later for those patients with missing data (n=17) on echocardiographic examinations at baseline. However, there were no differences in examination protocols, so this adjustment did not yield bias to the results.

6.3.1.2 Confounding or intermediate variable?

A confounder is a variable associated to both the exposure and the outcome, but does not comprise the causal pathway between the exposure and the outcome. If that is the case, then it is an intermediate variable, Figure 20. Depending on the research question asked, it is common to adjust for confounders in regression analyses, but show crude and unadjusted results for intermediate variables.

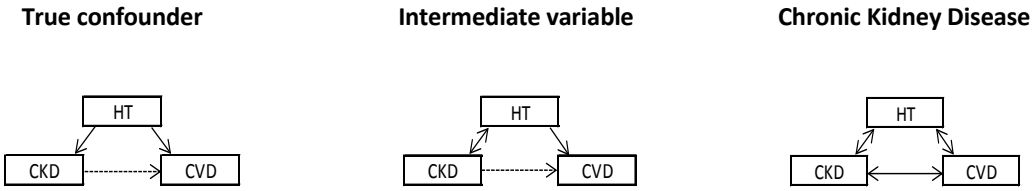


Figure 20. Difference between true confounder and intermediate variable and the relations in Chronic Kidney Disease. HT= Hypertension, CKD= Chronic Kidney Disease, CVD= Cardiovascular Disease.

The exposures of choice in the included studies were CKD, i.e. reduced renal function and the outcomes were biomarkers of CV risk in Paper I, alterations in LVMI, markers of diastolic function and/or cIMT in Paper II-III and markers for CKD-MBD in Paper IV. Unfortunately as demonstrated in Figure 20, it is not always easy to state a variable as a confounder or intermediate variable in CKD studies. For example, hypertension is known to be a considerable risk factor for CVD. Also, hypertension causes CKD, and furthermore, both CKD and CVD further worsen hypertension.

The question is then, which variables should be included in the regression analysis, and which should not. The statistical models in this thesis tried to adjust for as many covariates as possible known to be associated with both the exposure and the outcome.

In Paper I, the outcome of choice was biomarkers of CV risk. The regression analysis were adjusted for age, gender, lean versus non-lean (i.e. overweight or obese) and puberty, as these variables were known to affect the outcome (insulin or HOMA-IR). When adjusting for these variables, the true effect of CKD on insulin or HOMA-IR could be assessed.

In Paper II, age and ABP z-scores were included in all multivariate regression analyses due to potential confounding on the outcome LVMI or diastolic function markers. Also, those variables found to be associated with the outcome of choice in univariate analyses were included.

Based on the results from Paper II, models in Paper III were adjusted for age, gender, BMI z-scores, ABP z-scores, Albuminuria and GFR when analyzing CV outcome. These variables have all been independently associated with CV outcome in previous large studies in adults with CKD, and were clinically relevant to include in the model.

In Paper IV on the other hand, all variables previously shown to be associated with the outcome FGF23 and/or Klotho were included; age, GFR, Calcium, Phosphate, PTH and use

of Vitamin-D supplementation. In the analyses for CV outcome, the independent variables were tested in both univariate and multivariate models. Age, BMI and SBP z-scores and GFR were forced into the multivariate models due to potential confounding based on results from Papers II and III.

Importantly, in statistical models it is only possible to adjust for known variables. Unmeasured variables may still be of importance and obscure a true relationship.

6.3.1.3 Limitations

The major limitation in this thesis is missing data (Papers II-IV). It is foremost an issue regarding the echocardiographic and cIMT analyses as well as ABPMs. Fortunately with regard to biomarkers, GFR and office BP, there was high adherence to the original protocol. In Paper III, the problem of missing outcome data was solved by including those with missing data at baseline one year earlier or later. Also, multiple imputations on data for ABPM were used. Furthermore, the statistical model chosen was a mixed model, which is the best choice in cases of missing (at random) longitudinal data.

Also, it is important to consider the potential bias in the CKD group in Papers III-IV over time, during which 7 patients were transplanted during follow-up, which meant that those with the worst renal function dropped-out. This is exemplified when analyzing GFR longitudinally, as GFR was unchanged in the CKD group, while mean GFR declined significantly over follow-up in CKD-T patients with an annual rate of 2.2 ml/min/1.73m² ($p<0.001$), Paper III.

Furthermore, neither of our chosen reference groups was completely healthy. Nonetheless, they had normal renal function and in Papers I, III and IV, and they did not have present albuminuria. However, in Paper II two patients did have present albuminuria. While their urinary albumin to creatinine ratios was low, it is impossible to rule out the possibility that they had sub-clinically affected renal function.

In addition, the use of spot urine samples to assess albuminuria could be questioned. While this method has high specificity (≥97%), the sensitivity is poor as false negative samples can be encountered when urine samples are particularly dilute[264]. It would have been preferable to use urine albumin to creatinine ratio as recommended in the KDIGO 2012 guidelines, but these data were not available for all study participants. Furthermore, the use of a cut-off set at 20mg/L and reporting albuminuria as a categorical variable rather than a continuous variable could be discussed. In fact, previous meta-analyses from the general population show a linearly increased risk of CVD as urine albumin to creatinine ratio increases[241], which cannot be assessed using a categorical variable.

Finally, it is possible that the limited number of patients in these studies did not yield enough power to recognize true significant differences. However, as many patients as possible from this single-center unit were included within a feasible time frame.

7 CONCLUSIONS

• While there were no differences in vascular morbidity (carotid IMT), cardiac examinations revealed increased LVMI and signs of LV diastolic dysfunction. These were considered early markers of cardiac morbidity. Tissue Doppler imaging was more sensitive than pulse wave Doppler in detecting LV diastolic dysfunction using echocardiography.

• Traditional and uremic-related risk factors for CVD were commonly present in pediatric CKD and persisted following renal transplantation.

• Despite a low prevalence of ambulatory hypertension, even slightly elevated ambulatory blood pressure was an important risk factor for cardiac remodeling and diastolic dysfunction. Already a blood pressure just above the 50^{th} percentile was associated with increased risk for diastolic dysfunction, and therefore aiming for blood pressure targets in this range in antihypertensive treatment might be relevant.

• Other important risk factors were an increased BMI, low GFR and presence of albuminuria. Thus, overweight or obese patients as well as those experiencing deteriorating renal function and increasing albuminuria should undergo echocardiographic examinations to rule out possible subclinical CVD.

• Furthermore, while the patients included in this thesis demonstrate signs of anemia, altered glucose metabolism and dyslipidemia, only elevated cholesterol was associated with cardiac abnormalities as measured by tissue Doppler imaging (E/e´). Lifestyle changes and medication modifications might be important for these patients, but whether statin treatment is an alternative need to be further explored.

• FGF23 and Klotho are two novel markers of CKD-MBD. Their levels are altered early in the CKD progress and might also be important for the development of LV diastolic dysfunction in pediatric CKD-T patients.

8 FUTURE AND ONGOING RESEARCH

After writing this thesis it is clear that more research in this field is necessary. If funding will be available in the future it would be of great interest to follow the cohort included in Papers II-IV in this thesis into adulthood and to perform a 10 year follow-up. A new method for examining the heart is cardio magnetic resonance (CMR) imaging, enabling assessments of cardiac structure and function, atrial function, and myocardial tissue characterization including assessment of interstitial fibrosis[265]. To perform CMR imaging along with markers for CKD-MBD as well as markers of cardiac injury (e.g. NT-pro BNP) could yield additional information about this cohort.

Furthermore, we have data derived from dual-enery x-ray absorptiometry (DXA) measurements and it would be interesting to see if bone density is correlated to the FGF23-Klotho axis and CV outcome. Another interesting research field involves analyzing Klotho in renal biopsies and correlating these levels to serum Klotho, FGF23, GFR and CV data.

However, despite the fact that single center studies are easier to perform and allow the best control of the cohort studied, multicenter studies are important for generating information from large cohorts. It will be interesting to see future results generated from for example the American and Canadian CKiD (n≈600) and European 4C-cohorts (n≈700) with regard to CVD progression and predictive risk factors in pediatric CKD.

9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Njurarna har en central roll i kroppen, där den huvudsakliga funktionen är att utsöndra slaggprodukter i urinen samt kontrollera och reglera vätskebalans, blodtryck, saltbalans, blodets surhetsgrad, mineralisering av skelettet och blodbildning. Terminal njursvikt innebär att njurfunktionen inte är tillräcklig för att upprätthålla adekvat nivå av dessa funktioner och dialysbehandling eller njurtransplantation startas. Terminal njursvikt drabbar cirka 1000 vuxna och 15 barn och ungdomar per år i Sverige. Orsakerna till en sviktande njurfunktion skiljer sig mellan barn och vuxna, där barn oftare har medfödda förändringar i njurar och/eller urinvägar som orsak. Trots att njurarna får hjälp att utföra sina uppgifter i kroppen, är tyvärr riskerna för komplikationer stor. Vad gäller patienter med dialysbehandling finns en kraftigt ökad risk för dödlighet i såväl hjärt-kärlsjukdomar som infektioner. Även om risken minskar efter njurtransplantation, är dödligheten fortfarande förhöjd jämfört med friska jämnåriga.

Syftet med avhandlingen var att undersöka hur drabbade barn med nedsatt njurfunktion är av hjärt-kärlförändringar och identifiera potentiella riskfaktorer. I avhandlingen ingår fyra studier av totalt 104 barn som antingen har kronisk njursvikt eller är njurtransplanterade.

I delarbete I och II mättes olika inflammationsmarkörer, hemoglobin (blodvärde), blodfetter samt insulin och blodsocker. Njurfunktion, blodtryck och förekomst av proteinuri kontrollerades. Patienterna hade ökad förekomst av insulin-resistens samt förhöjda inflammations markörer och lågt hemoglobin, men det var inte kopplat till förändringar i hjärta eller kärl. Av de blodfetter som analyserades, var ett förhöjt kolesterol relaterat till en försämrad funktion i hjärtat. Ett förhöjt blodtryck samt förekomst av proteinuri var dessutom starkt kopplat till hypertrofi i hjärtat och/eller försämrad hjärtfunktion. Ultraljud av hjärtat med så kallad vävnadsdoppler var känsligare än traditionell metod; pulsvågs doppler, för att detektera tidiga förändringar.

Delarbete III och IV var uppföljningsstudier där en kohort om 30-31 barn med kronisk njursvikt samt 42-43 njurtransplanterade barn undersöktes årligen i tre år. Detta genererade max fyra mättillfällen per barn där ultraljud på hjärta och halskärl utfördes, samt dygnsmätningar av blodtryck, blodprover och njurfunktionsmätning undersöktes. I delarbete III framkom tydligt att såväl hypertrofi av hjärtmuskulatur som försämrad hjärtfunktion var associerat med förhöjt blodtryck. En ökning i blodtryck över tid medförde ytterligare förhöjd risk. En försämrad njurfunktion och förekomst av protein i urinen samt ung ålder var också viktiga för en försämrad funktion i hjärtat. I delarbete IV noterades att proteinet FGF23 och dess ko-faktor α-Klotho, som är viktiga för att reglera fosfat och vitamin-D balansen i kroppen, var associerade till försämrad diastolisk hjärtfunktion.

Sammanfattningsvis har barn med kronisk njursvikt och njurtransplanterade barn en betydande risk att utveckla förändringar i hjärtat, där den främsta riskfaktorn är förhöjt blodtryck. Huruvida dessa små förändringar är kopplade till att senare i livet utveckla symptomatisk hjärt-kärlsjukdom är ännu inte känt. Framtiden får utvisa om fler studier konfirmerar vårt fynd gällande möjlig association mellan FGF23-Klotho och hjärtfunktion.

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